Neisseria Meningitidis, Meningococcal Vaccines and Nutrition in Children in the African Meningitis Belt

A DISSERTATION SUBMITTED TO THE FACULTY OF THE UNIVERSITY OF MINNESOTA BY

Maria E Sundaram, MSPH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Advisers: Dr. Michael T. Osterholm, PhD & Dr. Nicole E. Basta, PhD

May 2018

Copyright Page

Acknowledgments

I gratefully acknowledge the support of the Frieda Martha Kunze Fellowship, which supported my dissertation work, and the support of my committee, my friends and my family, without whom the writing of this dissertation would not have been possible. I am especially thankful to my mentors Drs. Michael Osterholm, Edward Belongia, and Laura Coleman, as well as my grad-school wife, Yang Liu, and my esteemed colleagues Evan Sorley, Michelle Meyer, Coleman Drake, Bridget Griffith, Kimberly Bonner, and Dr. Becca Miller. I am indebted to Annie Lange, Pamela Andersen, Lauren Monteen, Louann Estresse Terveer, and Brady Sinho Rogers Hegberg for pep talks; to Faith Bruxa Hoenecke, Kelly Guerreira Schumann, Eric TDWG Wallin and Ira Guerreiro Weatherspoon for reminding me there is life outside of dissertating; to my father Dr. Rajah Sundaram, for encouraging both my curiosity and my impatience; and to Drs. J. Michael Oakes and Richard Maclehose for always reminding me I am terrible at science. I am extraordinarily lucky to have worked in a department chaired by Dr. Bruce Alexander, whose integrity and kindness provided a wonderful work environment; and to have worked with Khosi Nkosi and Laurel O'Neil, who fed me several pounds of chocolate and would not dream of letting me give up.

Dedication

I dedicate my dissertation to the many incredible female role models I have had in the course of my scientific career. To my unstoppable grandmother, Dr. Alamelu Sundaram, who began the Sundaram family women-in-science tradition by getting her PhD in chemistry in the 1950s; to my loving mother, Ruth Sundaram, who shows me how love is strength in everything that I do, especially science; to my incredible mentor Dr. Laura Coleman, who I trust more than myself; and to my brilliant advisor Dr. Nicole Basta, whose very first PhD student I am. I sincerely hope to follow in your footsteps.

Abstract

Introduction: Human nutrition plays an important role in immune function and protection against infectious diseases. Populations experiencing nutritional deficiencies may benefit less from vaccines (which require robust immune system function) and may be at increased risk of disease.

Methods: I identified populations living in the African meningitis belt and assessed potential relationships between 1) protein-energy undernutrition and meningococcal vaccine immunogenicity; 2) iron and vitamin A deficiency and long-term vaccine antibody persistence; and 3) iron status and risk of asymptomatic nasopharyngeal carriage of *Neisseria meningitidis* (Nm).

Results: Protein-energy undernutrition was not consistently significantly related to meningococcal vaccine immunogenicity in children 0-2 years old. However, increasing iron status was significantly related to a reduction in meningococcal vaccine-elicited antibody at 2 years post-vaccination. Finally, increasing iron status was significantly related to reduced odds of asymptomatic nasopharyngeal carriage of Nm in children 5-11 (for iron measured by serum ferritin) and 12-17 (for iron measured by soluble transferrin receptor) years old.

Discussion: This dissertation identifies potential relationships between iron status and meningococcal vaccine antibody persistence as well as the odds of Nm carriage. Further studies should assess these relationships in larger populations of children, at a greater number of time points, and consider additional iron biomarkers.

Table of Contents

List of Tables

List of Figures

Abbreviations

AGP, α-1-acid glycoprotein.

aOR, adjusted odds ratio.

CI, confidence interval.

CRP, C-reactive protein.

ELISA, enzyme-linked immunosorbent assay.

GGT, γ-glutamyl-transferase.

GMC, geometric mean concentration.

GMT, geometric mean titer.

HAZ, height-for-age Z-score.

HRP, horseradish peroxidase.

IgA, immunoglobulin A.

IgG, immunoglobulin G.

IgM, immunoglobulin M.

IQR, interquartile range.

MAP Study, MenAfriVac Antibody Persistence Study.

Nm, *Neisseria meningitidis*.

NmA, *Neisseria meningitidis* serogroup A.

ONPG, ortho-nitrophenyl-β-D-galactopyranoside.

OR, odds ratio.

PEU, protein-energy undernutrition.

RA, retinoic acid.

RBP, retinol binding protein.

RCD, reverse cumulative distribution.

rSBA, serum bactericidal antibody with rabbit complement.

SD, standard deviation.

sTfR, soluble transferrin receptor.

uOR, unadjusted odds ratio.

WAZ, weight-for-age Z-score.

WHO, World Health Organization.

WHZ, weight-for-height Z-score.

Introduction

Meningococcal meningitis is caused by *Neisseria meningitidis*, a Gram-negative bacterium (1) with 13 known serogroups, defined by differences in the structure of the bacterium's polysaccharide capsule (2, 3). Of these serogroups, six (A, B, C, W, X and Y) cause the majority of meningococcal disease worldwide (3-5). The disease burden is highest in Africa, with attack rates as high as 1,000 per 100,000 population; in other areas of the world, attack rates range from 0.3 to 3 per 100,000 population (6). The case fatality ratio has been estimated to be between 10% and 20% worldwide (7); similar estimates have been reported for recent meningococcal meningitis outbreaks in African countries (8). Survivors can suffer from permanent sequelae including deafness, cognitive impairment, seizures, and gangrene resulting in limb amputation (1, 9, 10). Meningococcal meningitis disease progresses rapidly and requires intensive supportive care, including administration of antibiotics and lumbar punctures that may be difficult to provide during epidemic conditions (1, 3). In addition to invasive infection, the bacterium is also capable of colonizing the upper respiratory tract (11), resulting in asymptomatic carriage lasting days or weeks.

Seasonal epidemics of meningococcal meningitis have been occurring for more than a century across the Sahel region of Africa; this area is called the "African meningitis belt" (12, 13). The African meningitis belt encompasses 22 countries and more than 400 million people (12), and has a high number of epidemics per year and higher rates of meningococcal meningitis overall,

compared to Western countries (3). Local epidemics of meningococcal meningitis in the meningitis belt seem to be seasonal (occurring during the dry season); large epidemics are cyclical in nature (occurring approximately every 5-8 years) (6).

Historically, outbreaks of meningococcal meningitis in the African meningitis belt have been reactively controlled using vaccination with polysaccharide vaccines (14-16), which contain antigens against bacterial capsular polysaccharides. However, reactive vaccination strategies are often too slow to control outbreaks effectively (14, 17); polysaccharide C, Y and W vaccines do not provide protection in children under 2 years; and the immunogenicity of polysaccharide A vaccines in young children is unclear (18). In order to better control epidemics of meningococcal meningitis, a new vaccine was developed against *N. meningitidis* serogroup A (NmA) (14). This vaccine, called MenAfriVac, contains NmA polysaccharide conjugated to a tetanus toxoid protein (19). MenAfriVac is safe (20) and efficacious (21), and provides antibody persistence for several years (22, 23). MenAfriVac vaccination campaigns have successfully reduced the incidence of meningitis caused by NmA (2, 24).

Although MenAfriVac immunogenicity is high, some studies have noted heterogeneity in immunogenicity and persistence of vaccine-elicited antibody according to demographic and immune factors (22, 25). This may leave some vaccinated groups more vulnerable to post-vaccination infection with NmA. MenAfriVac immunogenicity may vary depending on factors including age and

gender (25) and pre-vaccination levels of antibody (26). Studies on other vaccines have indicated that infant protein-energy and micronutrient undernutrition may also be associated with vaccine immunogenicity, though experts note that study results are conflicting depending on the setting, the pathogen, and the nutritional markers measured (27, 28).

Children with protein-energy undernutrition, a condition that results from a lack of sufficient energy and/or protein and leads to deficits in growth, are a population that is at increased risk of infectious disease morbidity and mortality (29). PEU is associated with poorer immune function and increased risk of infection (29, 30) in children. Infection in early childhood can impair the development of gut and lung epithelia, which increases the risk of future infection while also inhibiting the uptake of nutrients across gut epithelia (29). This inhibited uptake of nutrients results in further delays in epithelial development, further increasing the risk of infection. As a result, this interaction is termed a "vicious cycle" between infection and nutrition (31). Because PEU is considered to be an immunosuppressive state, it has been hypothesized that PEU may also negatively affect childhood immune response to vaccination (27, 28). Although this possibility has been investigated for many types of vaccines, including oral vaccines (27, 28), few studies have investigated this association for meningococcal meningitis vaccines (27). To our knowledge, no study has assessed the possible relationship between undernutrition and immune response to meningococcal conjugate vaccines (28).

In addition to PEU, low iron and low vitamin A are common in children in African meningitis belt countries (32, 33). Children with iron or vitamin A deficiency may be at increased risk for infection because both iron and vitamin A have been implicated in immune system function and child survival (29). Dendritic cells in the gut and lungs convert retinal (a derivative of vitamin A) to retinoic acid (RA, vitamin A's active form), identifying RA as a component of the immune protection of barrier sites (34). RA also aids the conversion of CD4+ T cells to induced regulatory T cells *in vitro*, and the conversion of CD4+ T cells to CD8+ T cells within intestinal epithelia (34). Meanwhile, iron is required for the survival of most bacterial and viral pathogens (35) and is therefore sequestered by the human immune system during times of acute infection, a process termed "nutritional immunity" (36). Iron also plays a role in natural host immunity in the absence of infection: iron availability modulates the balance between populations of Th1 and Th2 T helper cell subsets (37, 38), and is instrumental in the creation of toxic oxygen and nitrogen species that can kill microbes (39). Because iron and vitamin A are implicated in immune system function, vaccine immunogenicity and long-term antibody persistence (both of which require functional immune responses) in young children could vary according to iron or vitamin A status.

Finally, the probability of carriage of *N. meningitidis* may vary based on individual nutritional status. Other studies have indicated that *Plasmodium falciparum*, the parasite that causes malaria, may survive more easily in individuals with high iron levels (40). This observation regarding malaria and iron

has raised concerns about whether iron supplementation for anemic populations should be carried out in areas of the world with high malaria prevalence. Like *P. falciparum*, *N. meningitidis* requires iron to reproduce (41). So far, however, no studies have investigated whether iron levels are related to the risk of *N. meningitidis* infection or asymptomatic carriage. Since iron-deficiency anemia is also relatively common in the African meningitis belt (42), this question is of pressing concern. The prevalence of asymptomatic carriage of Nm is recognized as a predictive factor in meningococcal meningitis outbreaks (43, 44), but it has been noted that relatively little is known about the natural history of nasopharyngeal carriage of meningococcal meningitis (45, 46). A better understanding of the potential nutritional risk factors for Nm carriage could help contextualize the high variation in carriage prevalence and carriage type seen across the meningitis belt (45, 46).

The next steps in the control of meningococcal meningitis are therefore 1) to understand heterogeneity of the immune response to vaccination according to nutritional status, and 2) to elucidate the possible connections between nutrition and *N. meningitidis* carriage probability. So far, no study has assessed the impact of undernutrition on immediate or long-term immunogenicity of MenAfriVac, and no study has investigated a connection between iron undernutrition and *N. meningitidis* carriage. The analyses described in this dissertation seek to answer these questions.

Understanding the heterogeneity of immune response to vaccination and the potential association of MenAfriVac vaccination with increases in non-NmA serogroup incidence has important implications for current MenAfriVac vaccination campaigns and the future tailoring of vaccination campaigns for non-NmA serogroups of *N. meningitidis*.

Chapter 1: Meningococcal vaccines and protein-energy undernutrition in children in the African meningitis belt

Introduction

Meningococcal meningitis is a serious health problem in a region of Africa known as the African meningitis belt (12). In this area, young children experience a high burden of meningococcal disease (3). There are several vaccines available to prevent meningococcal disease and control outbreaks. These include PsACWY, a quadrivalent polysaccharide vaccine against *Neisseria meningitidis* serogroups A, C, W and Y; and PsA-TT, a polysaccharide-protein conjugate vaccine against *N. meningitidis* serogroup A. PsACWY is routinely used for outbreak control (2) and PsA-TT mass-vaccination campaigns have significantly reduced the burden of MenA disease in Africa since their introduction in 2010 (16). The World Health Organization recently issued a recommendation to incorporate PsA-TT into standard infant immunization schedules (47). Adding PsA-TT to infant immunization schedules means that all children, including vulnerable populations at increased risk of infection, will be eligible for vaccination.

Children with protein-energy undernutrition, a condition that results from a lack of sufficient energy and/or protein and leads to deficits in growth, are at increased risk of infectious disease morbidity and mortality (29). Protein-energy

undernutrition (PEU) can range from mild to severe and is diagnosed by comparing child-specific anthropometric values to corresponding WHO growth standards. PEU is prevalent in the countries of the African meningitis belt. Of children under 5 in urban Mali, approximately 21% have a weight-for-age value more than 2 standard deviations below the WHO reference mean, and 28% have a height-for-age value more than 2 standard deviations below the WHO reference mean (48). Similar estimates are seen in children under 5 in other meningitis belt countries, including Senegal (49), the Gambia (50), and Ghana (51). PEU is of special concern in areas with relatively higher risk of infectious disease such as the meningitis belt, because PEU is associated with poorer immune function and increased risk of infection (29, 30) in children. Infection in early childhood can impair the development of gut and lung epithelia, which increases the risk of future infection while also inhibiting the uptake of nutrients across gut epithelia (29). This inhibited uptake of nutrients results in further delays in epithelial development, further increasing the risk of infection. As a result, this interaction is termed a “vicious cycle” between infection and nutrition (31). Because PEU is considered to be an immunosuppressive state, it has been hypothesized that PEU may also negatively affect childhood immune response to vaccination (27, 28). Although this possibility has been investigated for many types of vaccines, including oral vaccines (27, 28), few studies have investigated this association for meningococcal meningitis vaccines (27). The few existing studies on this topic present conflicting findings, study only a short time-scale

post-vaccination (most studies measured post-vaccination antibody at 2 or 4 weeks post-vaccination only), and were largely conducted several decades ago with different vaccines than are currently used (52-55). Given the high prevalence of PEU in children living in the meningitis belt and the potential for PEU to limit immunological protection against infectious diseases, additional research is needed to investigate anthropometric growth indices and vaccine antibody response in the context of current meningococcal meningitis vaccines and to examine vaccine-elicited antibody on a longer time scale.

We investigated whether anthropometric growth indices in children living in the meningitis belt were associated with reduced meningococcal meningitis vaccine antibody response and long-term antibody persistence. We analyzed the relationship between growth indices and two different meningococcal vaccines. First, we investigated PsA-TT, a polysaccharide-protein conjugate vaccine. This vaccine is capable of eliciting a more robust immune response than polysaccharide-only vaccine because the conjugated protein is able to elicit a T cell-dependent response which primes immunological memory (14, 56), whereas polysaccharide-only vaccine antigens are only able to elicit a B cell immune response (19). Second, we investigated PsACWY, a quadrivalent polysaccharide vaccine that elicits an immune response using only polysaccharide. We analyzed vaccine antibody response and long-term antibody persistence according to conventional childhood growth indices (height-for-age, weight-for-height, and weight-for-age).

Materials and methods

Participants and data collection

This is a secondary analysis based on data from three clinical trials conducted as part of the Meningitis Vaccine Project. These trials were originally designed to quantify the safety and immunogenicity of PsA-TT in African children (57, 58). Our analysis uses clinical trial data from Malian children 12-23 months old (PsA-TT-002, conducted 2006-2008); Ghanaian children 14-18 weeks old (PsA-TT-004, conducted 2008-2011); and Malian children 9-12 months old (PsA-TT-007, conducted 2012-2013). A direct comparison of these studies can be found in **Table 1**. PsA-TT-002 randomized participants to receive PsA-TT, PsACWY, or a *Haemophilus influenzae* type b (Hib) conjugate vaccine in a 1:1:1 ratio (our analysis does not include children who received the Hib conjugate vaccine). In PsA-TT-004 and PsA-TT-007, all participants received PsA-TT (**Table 1**).

In each of these studies, participants provided blood samples and demographic information at pre-vaccination and 28-35 days post-vaccination. Additional blood samples and demographic information were provided at 10 months post-vaccination in PsA-TT-002, at 5 months post-vaccination in PsA-TT-004, and at 6 months post-vaccination in PsA-TT-007 (**Table 1**). Children were eligible for the original clinical trials if they were “free of obvious health problems”, per the child’s medical history and judgment of the trial investigator (57, 58).

Protocols from PsA-TT-002 and PsA-TT-003 are available online (57) and results of the primary trial aims have been published previously for PsA-TT-002 (57).

Laboratory methods and anthropometric measurement techniques

The methods to analyze serum samples collected from these three studies have been described elsewhere in detail (57, 58). Briefly, PsA-TT and PsACWY immunogenicity generated against *N. meningitidis* serogroup A was assessed just before vaccination and at several time points after vaccination, using group A serum bactericidal antibody with rabbit complement (rSBA). The rSBA reference strain was F8238; titers were expressed as the reciprocal of the final serum dilution. The lower limit of detection in PsA-TT-002 was an rSBA value of 4 (the LLQ has not yet been published for PsA-TT-004 and PsA-TT-007); samples with rSBA < 4 were assigned a value of 2 (57). Participant pre-vaccination weight, height (PsA-TT-002), length (PsA-TT-004 and PsA-TT-007), sex, and age, all collected after enrollment in the trials. We calculated weight-for-age (WAZ), weight-for-height (WHZ), and height-for-age (HAZ) Z-scores by comparing each child's measurements to the WHO 2006 Growth Standards (59). We compared children with the following Z-score categories: < -2 (the reference category); ≥ -2 and < -0; and ≥ 0.

Statistical analysis

Because rSBA antibody titers are results of a serial twofold dilution assay, rSBA values were $\log_2$-transformed. We used generalized linear regression analyses to investigate whether the change in antibody from pre-vaccination to 28-35 days post-vaccination was associated with categories of HAZ, WHZ, or WAZ. To account for potentially confounding differences between studies, we controlled for both the specific study and the country in which the study took place (a compound variable we called "study location").

We then used mixed linear regressions with random intercepts and random slopes to investigate whether post-vaccination antibody levels, and the rate of decline in antibody over the year post-vaccination, differed according to categories of HAZ, WHZ, or WAZ. In order to identify a potential association between anthropometric Z-score categories and the rate of decline in antibody over time, we incorporated interaction terms between Z-score categories and time since vaccination (in months) in the random-effects models. For these analyses, we assumed an exponential decline in antibody, meaning that we modeled a linear decline in log-transformed antibody. We excluded information about pre-vaccination antibody titer to model this linear decline. Children who received PsA-TT had antibody titer measured during at least 2 of the following time points: 1, 5, 6, and 10 months post-vaccination. Children who received PsACWY had antibody titer measured during at least 2 of the following time points: 1, 6, and 10 months post-vaccination (**Table 1**). Finally, in order to

provide additional context for results presented as log2 rSBA, the predicted mean rSBA was calculated for each month post-vaccination, using the Stata -margins- command.

Results

A total of 620 children were included in our analysis, including 199 (28.9%) from PsA-TT-002, 142 (20.6%) from PsA-TT-004, and 279 (40.6%) from PsA-TT-007 (**Table 1**). Of these participants, 310 (50.0%) were female; the mean (SD) age was 0.86 (0.45) years, and 521 (84.0%) received PsA-TT (**Table 2**). Pre-vaccination rSBA levels were low and increased immediately following vaccination in both PsA-TT and PsACWY recipients (**Table 2, Figure 1**), as has been reported previously (57).

In adjusted linear regression models, there was no evidence that HAZ (**Table 3**), WAZ (**Table 4**), or WHZ (**Table 5**) were associated with the change in PsA-TT-elicited antibody from pre-vaccination to 28-35 days post-vaccination. No association was identified between any anthropometric growth indices and the change in PsA-TT-elicited antibody from pre- to 28-35 days post-vaccination (**Tables 3-5**). In PsACWY recipients, WHZ ≥ -2 and < 0 (compared to WHZ < -2) was associated with an increase in $\log_2$ rSBA (difference: 2.45, 95% CI: 0.28 – 4.62) (**Table 4**), though this was not seen for other growth indices.

In adjusted mixed linear regression models with random intercepts and random slopes, increasing time post-vaccination was associated with a decline in

rSBA for both PsA-TT and PsACWY recipients, as expected (**Tables 3-5**). Nevertheless, interaction terms between HAZ, WHZ or WAZ and time post-vaccination (in months) were not found to be associated with rSBA in PsA-TT or PsACWY recipients (**Tables 3-5, Figure 2**). In these models, there was an association between WHZ ≥ -2 and < 0 (compared to WHZ < -2) and increased PsA-TT elicited antibody (difference in $\log_2$ rSBA: -1.03, 95% CI: -1.94, -0.12) (**Table 4**), and an association between WAZ ≥ -2 and < 0 (compared to WHZ < -2) (difference in $\log_2$ rSBA: 1.04, 95% CI: 0.07, 2.01) (**Table 5**). These results were not seen for PsACWY recipients.

The predicted mean rSBA for each anthropometric growth index was similar among PsA-TT recipients (rSBA range at 1 month post-vaccination: 1789.1 – 3224.8; rSBA range at 12 months post-vaccination: 62.1 – 412.4), and did not vary by growth index (**Figure 2**). The predicted mean rSBA for each anthropometric growth index was similar among PsACWY recipients (average rSBA at 1 month post-vaccination: 80.9 – 270.6; average rSBA at 12 months post-vaccination: 24.7 – 143.6), and did not vary by growth index (**Figure 2**).

Discussion

This analysis did not identify an association between HAZ, WHZ, or WAZ and the difference in rSBA antibody from pre- to 28-35 days post-vaccination with PsA-TT, but did identify an association between increasing WHZ and increasing rSBA from pre- to 28-35 days post-vaccination with PsACWY. Our analysis did

not identify an association between HAZ and PsA-TT antibody in a longitudinal analysis in the year following vaccination. Our analysis identified a potential association between lower WHZ and higher PsACWY-elicited antibody, and higher WAZ and higher PsACWY-elicited antibody, in a similar longitudinal analysis using random effects models. In these longitudinal analyses, neither PsA-TT recipients nor PsACWY recipients' post-vaccination antibody levels were significantly related to interaction terms between WAZ and time since vaccination. Results from this analysis do not consistently support the hypothesis that lower anthropometric growth indices are related to reduced PsA-TT or PsACWY antibody response, or lower antibody persistence, in children living in the African meningitis belt.

Our findings are similar to two previous studies conducted in Nigeria on the immune response to meningococcal polysaccharide vaccine published in the 1980s. In one study, the antibody response to group C meningococcal polysaccharide vaccine did not differ according to different deciles of weight-for-age, height-for-age, and arm circumference-for-age measures (although children with severe, "obvious" clinical malnutrition were excluded from the study) (54); in the other, the antibody response to several vaccines (including group A and group C meningococcal vaccine) did not differ according to weight-for-age measurements standard at the time (55). Our findings differ from another 1980s study on the immunological response to meningococcal polysaccharide vaccine among well-nourished and undernourished children in Nigeria, which found

reduced meningococcal A and meningococcal C-specific IgM, IgG and IgA responses to meningococcal polysaccharide vaccine in undernourished children (53). However, that study included a relatively small number of undernourished children (n = 14), the definition of undernourishment was not described, and the study was published 28 years before the introduction of PsA-TT in the African meningitis belt in 2010, during a time when standard analytic methods for nutrition and immunogenicity differed from current protocols. Furthermore, it is difficult to draw direct comparisons between immunoglobulin titer (reported in the study mentioned) and rSBA titers (reported in our analysis).

Among several comparisons, our analysis identified one statistically significant relationship between WAZ ≥ -2 and < 0, and increased rSBA during a year after vaccination with PsACWY. This result should be interpreted with caution for the following reasons: it was not replicated in analyses with PsA-TT, or with any other anthropometric value; the confidence interval for the statistically significant estimate was broad and the upper bound neared 1 (95% CI: 1.02 – 3.40); and an interaction term between WAZ and time since vaccination was not significantly related to vaccine antibody. In order to conclusively identify a relationship between increasing WAZ and decreasing antibody from PsACWY, additional studies specifically designed to investigate this question are necessary.

This study has several limitations. First, because this analysis incorporates information on vaccine antibody response and anthropometrics from

four clinical trials which took place in three different countries, and because the age of eligible participants differed slightly by study, there was random variation in participant anthropometrics and baseline antibody level by study and country. Despite the potential for unmeasured confounding, inclusion and exclusion criteria were otherwise similar between studies (57, 58), and all followed similar protocols. Additionally, we have accounted for potential between-study variation by including a regression covariate to describe study and country.

Second, children were excluded from the original clinical trials if they had "obvious health problems" (53). Therefore, although nutritional status was not a direct component of the study inclusion or exclusion criteria, this criterion may have resulted in the exclusion of some children with undernutrition. Recent estimates of HAZ < -2 in children 0-5 years old across Mali, Senegal, Ghana, and The Gambia are between 15.5% and 38% (48-51); estimates of WHZ < -2 are between 13% (49) and 17% (60), and estimates of WAZ < -2 are between 6.2% (60) and 26% (50). However, the prevalences of WHZ <-2 and WAZ <-2 in this analysis are similar to prevalence estimates reported for the general population, but the prevalence of HAZ <-2 in this analysis is slightly lower than reported for the overall population.

Finally, the longitudinal component of this analysis made use of several time points post-vaccination. However, these time points were not shared by all studies that comprised the analysis; the models used assume exponential decay of antibody over time; and post-vaccination time points were only included up to

10 months after vaccination. Future analyses would benefit from including additional time points post-vaccination and for a longer period of time (for example, at each month post-vaccination for several years), for higher analytic granularity.

Conclusion

This analysis did not identify a consistent relationship between protein-energy undernutrition and reduced vaccine antibody response or antibody persistence of PsA-TT or PsACWY in children living in the African meningitis belt. Future studies should consider measuring antibody titers at additional time points post-vaccination, and for longer periods of time, to determine if the rate of antibody waning over a period of several years is associated with nutritional status.

Acknowledgments

The authors gratefully acknowledge the work of the trial coordinators and field workers, the laboratory technician staff at Public Health England and NutriSurvey, and the contributions of the study participants themselves. The authors are additionally grateful for the scientific input and expertise of Dr. Ellen Demerath, PhD.

Table 1. Number of participants and timing of post-vaccination blood sample collection, by study.

Study name	Country	Partici-pants (N)	Partici-pant ages	Years of study	Study vaccines	Post-vaccination timepoints
PsA-TT-002	Mali	199	12-23 months old	2006-2008	PsA-TT, PsACWY, and Hib conjugate vaccine[a] (random-ized in 1:1:1 ratio)	• 1 month • 10 months
PsA-TT-004	Ghana	142	14-18 weeks old	2008-2011	PsA-TT only	• 1 month • 5 months
PsA-TT-007	Mali	279	9-12 months old	2012-2013	PsA-TT only	• 1 month • 6 months

[a] Only children who received PsA-TT or PsACWY were included in this analysis.

Table 2. Demographic and anthropometric characteristics of study participants.

Characteristic	PsA-TT recipients (N = 521)	PsACWY recipients (N = 99)
Age: mean (SD)	0.75 (0.39	1.42 (0.30)
Female sex: N (%)	265 (50.9)	45 (45.5)
Study and study site: N (%)		
PsA-TT-002, Mali	100 (19.2)	99 (100)
PsA-TT-004, Ghana	142 (27.3)	0 (0)
PsA-TT-007, Mali	279 (53.6)	0 (0)
rSBA GMT (95% CI)		
Pre-vaccination	2.8 (2.5 – 3.2)	4.5 (3.0 – 6.8)
28-35 days post-vaccination	3297.4 (2822.3 – 3852.7)	200.4 (112.1 – 358.2)
Anthropometric measurements[a]		
HAZ categories: N (%)		
Z-score <-2	20 (3.8)	16 (16.2)
Z-score ≥ -2 and < 0	274 (52.6)	51 (51.5)
Z-score ≥ 0	227 (43.6)	32 (32.3)
WHZ categories: N (%)		
Z-score < -2	67 (12.9)	20 (20.2)
Z-score ≥ -2 and < 0	308 (59.1)	62 (62.6)
Z-score ≥ 1	146 (28.0)	17 (17.2)
WAZ categories: N (%)		
Z-score < -2	44 (8.5)	18 (18.2)
Z-score ≥ -2 and < 0	338 (64.9)	65 (65.7)
Z-score ≥ 0	139 (26.7)	16 (16.2)
HAZ: mean (SD)	-0.19 (1.11)	-0.90 (1.26)
WHZ: mean (SD)	-0.63 (1.17)	-1.00 (1.33)
WAZ: mean (SD)	-0.56 (1.04)	-1.01 (1.16)

[a] Anthropometric measurements are measured in Z-scores. A Z-score of 0 indicates that the value for an individual is equal to the reference value mean; a Z-score less than 0 indicates that the value for an individual is lower than the reference value mean.

Table 3. The difference in rSBA from pre- to post-vaccination with PsA-TT and PsACWY, and the relationship between height-for-age and decline in antibody over time. [a]

Characteristic	PsA-TT			PsACWY		
	rSBA difference	95% CI		rSBA difference	95% CI	
Model 1: Antibody difference from pre- to 28-35d post-vaccination						
Z-score < -2 (ref)	--	--	--	--	--	--
Z-score ≥ -2 and < 0	0.16	-1.21	1.52	0.14	-2.36	2.63
Z-score ≥ 0	0.42	-0.97	1.80	0.36	-2.43	3.16
Model 2: Antibody decline over time post-vaccination[b]						
Z-score < -2 (ref)	--	--	--	--	--	--
Z-score ≥ -2 and < 0	-0.37	-1.77	1.03	0.17	-1.87	2.20
Z-score ≥ 0	0.06	-1.35	1.47	1.00	-1.26	3.26
Time since vaccination (in months)	**-0.50**	**-0.74**	**-0.26**	-0.12	-0.34	0.09
Time*Z-score < -2	--	--	--	--	--	--
Time*Z-score ≥ -2 and < 0	0.23	-0.02	0.48	-0.01	-0.26	0.24
Time*Z-score ≥ 0	0.12	-0.14	0.37	0.03	-0.24	0.29

[a] Each model shown is a separate regression model accounting for participant age, sex, study and study site as potential confounders. A value of 1.2 indicates a 20% increase in rSBA compared to the reference category or with a 1-unit change in the covariate of interest; a value of 0.8 indicates a 20% decrease in rSBA.

[b] Model 2 is a mixed-effects regression with random intercept and random slope, assuming an exchangeable correlation matrix.

Table 4. Linear regressions on the change in rSBA from pre- to post-vaccination with PsA-TT and PsACWY, according to weight-for-height, and the relationship between weight-for-height and decline in antibody over time. [a]

Characteristic	PsA-TT			PsACWY		
	rSBA difference	95% CI		rSBA difference	95% CI	
Model 1: Antibody difference from pre- to 28-35d post-vaccination						
Z-score < -2 (ref)	--	--	--	--	--	--
Z-score ≥ -2 and < 0	-0.59	-1.40	0.23	2.45	0.28	4.62
Z-score ≥ 0	-0.76	-1.67	0.14	1.97	-0.88	4.82
Model 2: Antibody decline over time post-vaccination						
Z-score < -2 (ref)	--	--	--	--	--	--
Z-score ≥ -2 and < 0	-0.51	-1.33	0.31	1.11	-0.77	2.99
Z-score ≥ 0	**-1.03**	**-1.94**	**-0.12**	-0.16	-2.52	2.21
Time since vaccination (in months)	**-0.31**	**-0.44**	**-0.18**	-0.13	-0.33	0.07
Time*Z-score < -2	--	--	--	--	--	--
Time*Z-score ≥ -2 and < 0	-0.05	-0.19	0.10	0.01	-0.22	0.24
Time*Z-score ≥ 0	0.03	-0.13	0.20	0.02	-0.26	0.31

[a] Each line shown is a separate regression model accounting for participant age, sex, study and study site as potential confounders. Positive values indicate an increase in antibody from pre- to post-vaccination associated with the anthropometric value.

Table 5. Linear regressions on the change in rSBA from pre- to post-vaccination with PsA-TT and PsACWY, according to weight-for-age, and the relationship between weight-for-age and decline in antibody over time. [a]

Characteristic	PsA-TT			PsACWY		
	rSBA difference	95% CI		rSBA difference	95% CI	
Model 1: Antibody from pre- to 28-35d post-vaccination						
Z-score < -2 (ref)	--	--	--	--	--	--
Z-score ≥ -2 and < 0	0.66	-0.31	1.62	2.23	-0.08	4.55
Z-score ≥ 0	0.36	-0.69	1.42	2.78	-0.32	5.87
Model 2: Antibody decline over time post-vaccination						
Z-score < -2 (ref)	--	--	--	--	--	--
Z-score ≥ -2 and < 0	**1.04**	**0.07**	**2.01**	0.44	-1.65	2.53
Z-score ≥ 0	0.54	-0.52	1.60	0.35	-2.41	3.10
Time since vaccination (in months)	**-0.19**	**-0.34**	**-0.04**	-0.15	-0.38	0.07
Time*Z-score < -2	--	--	--	--	--	--
Time*Z-score ≥ -2 and < 0	-0.16	-0.32	0.00	0.06	-0.19	0.31
Time*Z-score ≥ 0	-0.14	-0.33	0.05	-0.06	-0.38	0.27

[a] Each line shown is a separate regression model accounting for participant age, sex, study and study site as potential confounders. Positive values indicate an increase in antibody from pre- to post-vaccination associated with the anthropometric value.

Figure 1. RCD curves of rSBA titers according to anthropometric growth indices and vaccine received.

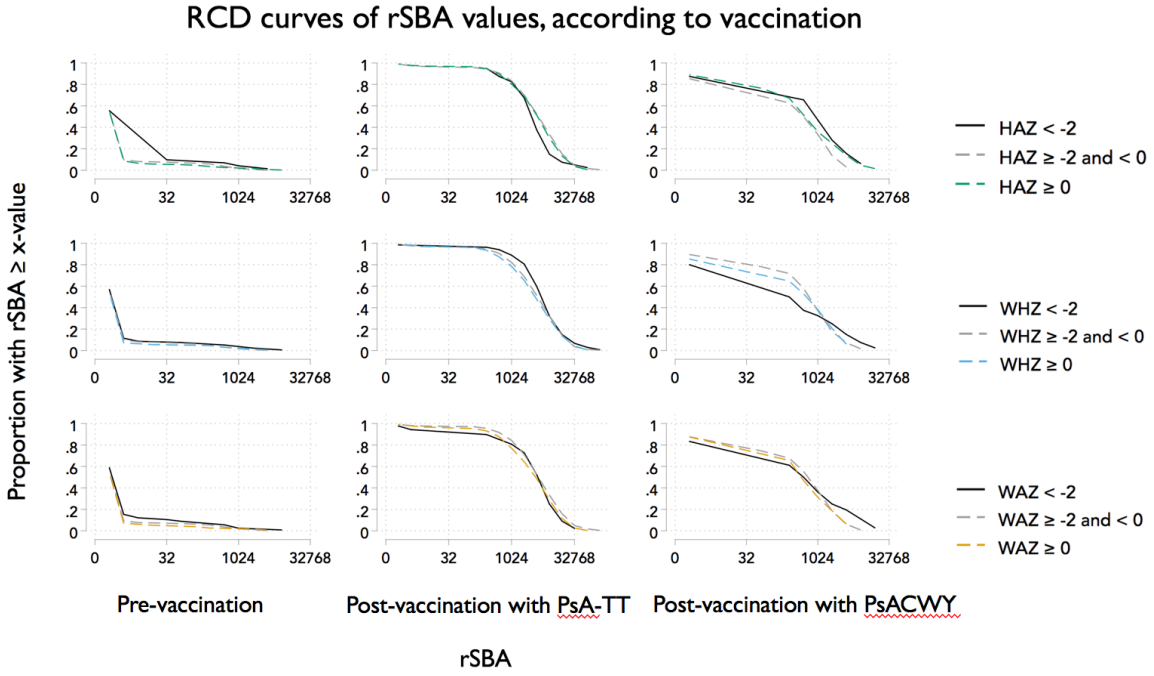
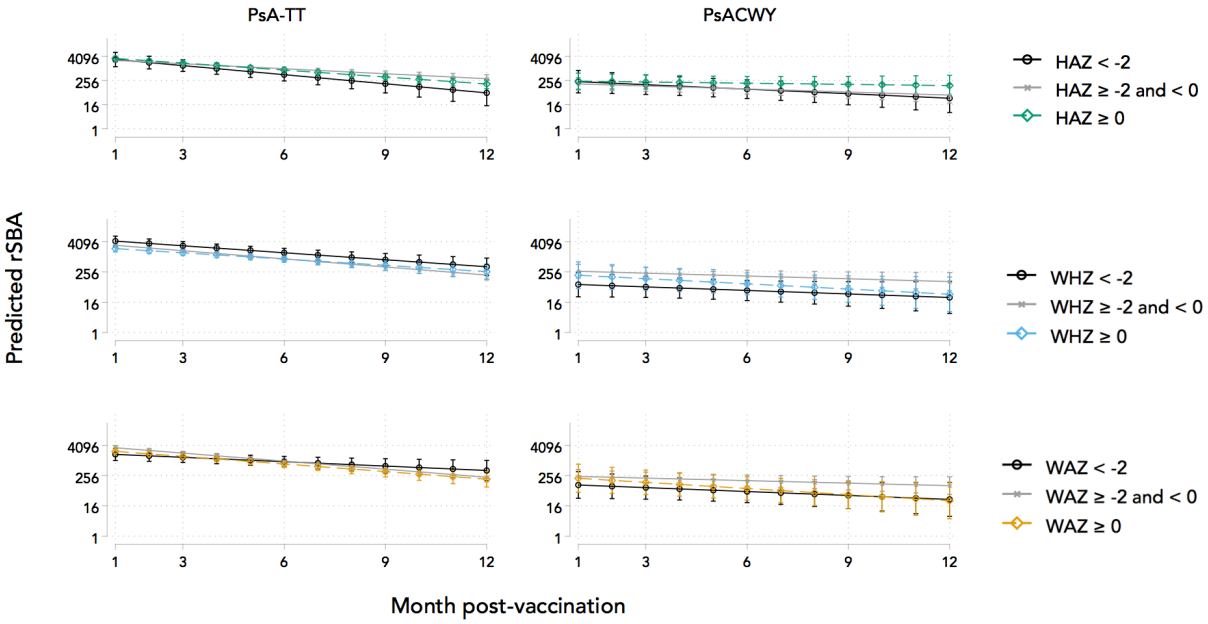


Figure 2. Predicted means of rSBA for PsA-TT and PsACWY recipients, over a year post-vaccination.



Chapter 2: Meningococcal vaccine antibody persistence and the relationship to iron and vitamin A

Introduction

Meningococcal meningitis caused by the bacterium *Neisseria meningitidis* has historically been a serious public health problem in the African meningitis belt (7, 12), especially in young children (3). MenAfriVac, a polysaccharide-protein conjugate vaccine to prevent meningococcal disease (including meningitis) caused by *N. meningitidis* serogroup A (NmA), is safe (20) and effective (21), and has successfully reduced the burden of meningococcal A meningitis in Africa (16). After several large-scale vaccination campaigns, the World Health Organization has now issued a recommendation to incorporate MenAfriVac into standard infant immunization schedules and administer the vaccine at 9-18 months of age (47). Clinical trials have demonstrated that MenAfriVac is highly immunogenic in healthy infants (57), but adding MenAfriVac to infant immunization schedules means that all children, including vulnerable populations at increased risk of infection, will be eligible for vaccination.

One of these potentially vulnerable populations is children with low levels of essential vitamins and minerals such as iron and vitamin A. Low iron and low vitamin A are common in children in African meningitis belt countries (32, 33); in Mali alone, it is estimated that 61% of children under 5 experience moderate or

severe iron-deficiency anemia (61) and greater than 90% of children are deficient in vitamin A (62). Children with iron or vitamin A deficiency may be at increased risk for infection because both iron and vitamin A have been implicated in immune system function and child survival (29). In a landmark cluster-randomized trial of vitamin A supplementation in 1986, vitamin A deficiency was associated with child mortality (63). More recently, in a recent systematic review and meta-analysis, vitamin A supplementation was found to be associated with reductions in childhood morbidity and mortality from diarrhea and measles (64); and in a randomized controlled trial in Indonesia, supplementation of vitamin A in both vitamin A-sufficient and vitamin A-deficient children was associated with increased immunogenicity for tetanus toxoid vaccine (65). Similarly, low iron status was associated with reduced antibody titers for tetanus toxoid vaccine and respiratory syncytial virus vaccine in a national cross-sectional study on nutrition and health in Ecuador (66). Conversely, however, supplementation of iron has been associated with increased risk of malaria (67), tuberculosis (68), and other infections (69), and iron deficiency appears to be protective against malarial infection in malaria-endemic areas (70).

These proposed relationships between immune function and iron or vitamin A are supported by identified biological mechanisms. Dendritic cells in the gut and lungs convert retinal (a derivative of vitamin A) to retinoic acid (RA, vitamin A's active form), identifying RA as a component of the immune protection of barrier sites (34). RA also aids the conversion of CD4+ T cells to induced

regulatory T cells *in vitro*, and the conversion of CD4+ T cells to CD8+ T cells within intestinal epithelia (34). Meanwhile, iron is required for the survival of most bacterial and viral pathogens (35) and is therefore sequestered by the human immune system during times of acute infection (36). Iron also plays a role in natural host immunity: iron availability modulates the balance between populations of Th1 and Th2 T helper cell subsets (37, 38), and is instrumental in the creation of toxic oxygen and nitrogen species that can kill microbes (39).

Because iron and vitamin A are implicated in immune system function, vaccine immunogenicity and long-term antibody persistence (both of which require functional immune responses) in young children could vary according to iron or vitamin A status. Given the potentially complex relationships between iron and vitamin A and immune system function, and the population-level deficiency in iron or vitamin A in the African meningitis belt, a better understanding of the potential associations between iron or vitamin A status and the immune response to meningococcal protein-conjugated polysaccharide vaccines (such as MenAfriVac) is needed. Therefore, we analyzed whether serum biomarkers of iron or vitamin A were associated with the long-term persistence of anti-MenA antibody in children in the meningitis belt. Specifically, we investigated whether iron or vitamin A status is associated with persistence of anti-MenA antibody at 2 years post-vaccination with MenAfriVac, and whether iron or vitamin A status is associated with change in anti-MenA antibody levels from 2 to 3.5 years post-vaccination.

Methods

Study population and enrollment

From December 12, 2010 to January 11, 2011, a MenAfriVac vaccination campaign was administered in Bamako, Mali, to individuals 1-29 years of age (71). The campaign was estimated to have covered more than 99% of eligible individuals living in the Banconi district of Bamako (72). Two years later in December 2012 – January 2013, in a study led by investigators now at the University of Minnesota in collaboration with researchers at the Center for Vaccine Development-Mali, individuals living in Banconi who had been eligible for vaccination during the campaign were recruited for a longitudinal cohort study to assess the persistence of MenAfriVac-elicited antibody (the MenAfriVac Antibody Persistence, or MAP, Study) (22). The methods of this study have been described previously (22); briefly, individuals were selected in December 2012 from an existing demographic surveillance system using a household-based, age-stratified random sampling design. Participants were eligible if the demographic surveillance system indicated they had been 1-29 years old and had been living in Banconi at the time of the vaccination campaign, had not participated in a MenAfriVac clinical trial, and had no acute or chronic health conditions. Study enrollees were re-contacted for a follow-up visit in May 2014. At both visits, participants provided demographic information and a blood sample. We restricted this analysis to individuals aged 1-5 years on December 1,

2010 at the time of vaccination, meaning individuals were 3-8 years old at the time of the first cohort visit in December 2012 – January 2013, and 4-9 years old at the time of the second cohort visit in May 2014.

Laboratory methods

The procedures for processing blood samples to extract the serum and quantify these markers of immunity have been described previously (22). Briefly, up to 8.5mL of blood was collected from each individual; the serum was extracted from the blood, divided into aliquots, and stored at -80°C before shipment to the Vaccine Evaluation Unit at Public Health England (Manchester, UK). The sera were assessed to quantify the complement-mediated serum bactericidal antibody titers, using baby rabbit complement (rSBA). The NmA reference strain used was F8238 (A:P1.20,9) (73). Serum samples were also tested for serum ferritin, soluble transferrin receptor (sTfR), retinol binding protein (RBP), and C-reactive protein (CRP) using a multiplex sandwich ELISA with HRP-coupled antibodies at the NutriSurvey lab (Wilstätt, Germany) (74).

In this analysis, serum ferritin and sTfR were used to assess iron status according to WHO guidelines (75). Serum ferritin is an acute phase protein, meaning its blood concentrations increase during acute infection, regardless of dietary iron status. In the absence of infection, levels of ferritin in the blood correlate with liver iron stores. sTfR levels are less affected by acute infection (75) and reflect availability of iron in the bone marrow. Higher blood

concentrations of sTfR reflect poorer iron status. CRP and AGP are acute phase proteins that mark acute and convalescent stages of infection, respectively.

Statistical analysis

Our primary aims were to assess 1) whether iron or vitamin A status at 2 years post-vaccination is associated with persistence of antibody at 2 years post-vaccination, and 2) whether iron or vitamin A status at 2 years post-vaccination is associated with change in antibody from 2 to 3.5 years post-vaccination.

In descriptive analyses, we defined iron deficiency as < 12μg/L for children < 5 years old, < 15 μg/L for children ≥ 5 years old, and < 30μg/L for children of any age who had CRP > 5mg/L (76). We defined RBP deficiency as < 0.75μmol/L, abnormal sTfR as > 8.3mg/L, high CRP as > 5mg/L, and high AGP as > 1mg/L, per assay guidelines (74). In regression models, serum ferritin, sTfR, and RBP were analyzed continuously; we $\log_{10}$ transformed ferritin and sTfR because the underlying distributions of both were right-skewed. rSBA was $\log_2$ transformed because it is a twofold serial dilution assay.

To address our primary aims, we fit separate linear regression models to assess the relationship between rSBA values and serum ferritin, sTfR, or RBP levels. Regression models controlled for age and sex as potential confounders, and included CRP and AGP as markers of concurrent acute or convalescent infection. Models also included information on child anthropometric growth as a potential confounder. Poor anthropometric growth can co-occur with deficiency in

iron or vitamin A (77, 78) and may have a separate potential effect on immune system function (79). We used participant weight, length/height, sex, and age to calculate weight-for-age (WAZ) and height-for-age (HAZ) Z-scores, using WHO child growth reference values (59). One child with a calculated HAZ > 5 standard deviations from the mean was excluded from the analysis, as it is likely this calculated value was a result of measurement error.

After obtaining regression coefficients for log-transformed values (log_2 rSBA), coefficients were back-transformed (2^{β}) to obtain fold changes for raw antibody values (keeping in mind that a difference in log_2 values translates to a ratio in raw values). To aid in interpretation of model results, the marginal means of raw rSBA were also calculated for continuous values and quintiles of serum ferritin, sTfR, and RBP, after controlling for confounders, using the -margins- command in Stata. Additionally, the same predicted marginal means of raw rSBA were graphed according to continuous values of serum ferritin, sTfR, and RBP. All analyses were performed using Stata 14 and Stata 15 (College Station, TX).

Results

There were 201 individuals who were enrolled in December 2012 – January 2013, approximately two years after the December 2010 MenAfriVac vaccination campaign. After excluding 1 individual with an unrealistic HAZ, the total analytic sample was 200. Of these individuals, 165 (82.5%) were successfully followed to 3.5 years post-vaccination. All individuals in this analysis

reported having received MenAfriVac during the 2010 campaign and were 1-5 years old at the time of vaccination; none reported ever having meningitis.

Of the 200 participants, 103 (51.5%) were female, and the mean (SD) age at 2 years post-vaccination was 6.0 (1.3) years. Demographic characteristics were similar between the participants enrolled at 2 years post-vaccination and the subset of the participants who were successfully followed to 3.5 years post-vaccination (**Table 6**). Most individuals had serum ferritin, sTfR, and vitamin A values within healthy ranges, though 30% of individuals had low sTfR at 2 years post-vaccination (**Table 6**). MenA rSBA geometric mean titer (GMT) was 2870.9 (95% CI: 2517.2 – 3274.4) at 2 years post-vaccination and significantly declined to 1686.1 (95% CI: 1416.0 – 2007.8) at 3.5 years post-vaccination.

In linear regression analyses on participants at 2 years post-vaccination, there was a 60% reduction in rSBA (95% CI: 19% - 80% decrease) for each unit increase in $\log_{10}$ serum ferritin (**Table 7**). Associations between $\log_{10}$ sTfR or RBP and rSBA were not identified at 2 years post-vaccination (**Table 7**). Log_{10} ferritin, $\log_{10}$ sTfR and RBP were not found to be associated with the change in rSBA from 2 to 3.5 years post-vaccination (**Table 8**).

Calculated marginal means of raw rSBA at quintiles of raw serum ferritin reflected the association between log serum ferritin and rSBA identified in linear regression analyses: higher marginal mean rSBA values were seen for lower quintiles of serum ferritin (**Table 9, Figures 7-9**). As in linear regression analyses, marginal means of raw rSBA were not subject to substantial change

among quintiles of sTfR or RBP (**Table 9**). Similarly, marginal means of the fold change in rSBA from 2 to 3.5 years post-vaccination did not appear to change among quintiles of serum ferritin, sTfR, or RBP (**Table 10, Figures 10-12**).

Discussion

Our analysis identified an association between increasing log ferritin and decreasing rSBA at 2 years post-vaccination, supported further by an analysis which calculated marginal means of rSBA at 2 years post-vaccination according to serum ferritin quintiles. Our analysis did not identify associations between log ferritin, log sTfR, and RBP and the change in rSBA from 2 to 3.5 years post-vaccination with MenAfriVac.

Previous studies on diphtheria and tetanus vaccine in India (80) and Ecuador (66), and a study on 23-valent pneumococcal vaccine in Gambian children (81), identified no consistent association between vitamin A deficiency and antibody response to vaccines. However, a randomized controlled trial in Indonesia identified higher IgG responses to diphtheria-tetanus-pertussis vaccine in children who received vitamin A supplementation, compared to those who did not (65). Our analysis did not identify a relationship between retinol binding protein and either rSBA or IgG ELISA response to MenAfriVac; however, our study measured vitamin A using retinol binding protein (the Indonesian study stated that “plasma vitamin A”, assumed to be retinol, was measured). Furthermore, we measured antibody outcomes 2 and 3.5 years after vaccination

(the Indonesian study measured antibody outcomes three weeks after vaccination).

A previous study indicated that Ecuadorian children with iron deficiency may have lower antibody titers to diphtheria and tetanus after vaccination (66). It has been noted that further studies on this topic are sparse (27). Our analysis adds to the existing literature by describing the relationship between vaccine antibody and iron and vitamin A in a population-representative sample of otherwise healthy children rather than focusing specifically on children with iron or vitamin A deficiency.

Our analysis identified a decline in rSBA antibody titer from 2 to 3.5 years post-vaccination. However, at 3.5 years post-vaccination, rSBA antibody titers were still high, and the change in rSBA antibody titer from 2 to 3.5 years was not related to iron or vitamin A. Future studies assessing factors associated with the magnitude of post-vaccination anti-MenA antibody should consider accounting for potential rSBA decline over time.

Our analysis included CRP and AGP to account for changes in serum ferritin that may occur during times of acute or chronic infection and inflammation (75). Although the timing and magnitude of serum ferritin changes during infection most closely resemble changes in CRP (not AGP), some WHO recommendations suggest including both CRP and AGP in analyses of serum ferritin if both measures are available (75). Likelihood ratio tests comparing each

model reported in this paper with and without AGP as a covariate did not identify statistical differences between these models (results not shown).

This analysis is subject to several limitations. First, most children in this study had high levels of anti-NmA antibody both at 2 and 3.5 years post-vaccination. It is therefore unclear whether a relationship between serum ferritin and MenAfriVac-elicited rSBA antibody is likely to have an immediate impact on protection against meningococcal meningitis. Second, our analysis was set up to identify associations between iron or vitamin A and antibody at two discrete time points post-vaccination. As such, this analysis is not able to determine the cumulative effect of long-term iron or vitamin A deficiency on MenAfriVac antibody persistence over time, nor is it able to identify pre-vaccination factors which could affect the immunogenicity of MenAfriVac. Including additional pre- and post-vaccination measurements of MenAfriVac antibody and iron or vitamin A in future studies would aid in identifying long-term trends and potential relationships between iron or vitamin A and MenAfriVac antibody.

Finally, this was a secondary analysis intended for hypothesis generation. As such, it includes a relatively small number of individuals, and may lack statistical power to identify small associations between iron and vitamin A status and MenAfriVac-elicited antibody. Results from this analysis should therefore be interpreted as suggestions for directing future research.

Despite these limitations, this exploratory analysis identifies a potential relationship between serum ferritin and MenAfriVac antibody persistence that has

not been described before in the literature. Results from this analysis add to the existing literature on iron status and vaccine response, and suggest future studies to identify the potential longitudinal effects of iron status and severe iron deficiency on post-vaccination persistence of antibody.

Conclusions

This analysis identified an association between log ferritin and rSBA at 2 years post-vaccination. However, this analysis did not identify an association between log sTfR or RBP and rSBA at 2 years post-vaccination. This analysis also did not identify an association between log ferritin, log sTfR, or RBP and change in rSBA from 2 to 3.5 years post-vaccination. To our knowledge, this is the first study to investigate possible relationships between iron and vitamin A status and antibody persistence from meningococcal polysaccharide-protein conjugate vaccine. Future studies should be undertaken to confirm these findings in larger cohorts and to identify the potential longitudinal effects of iron status on post-vaccination persistence of antibody.

Table 6. Descriptive statistics of children originally enrolled in 2012 and the subset of the originally enrolled children that were followed to a second visit in 2014.

Characteristic	Initial visit: 2 years post-vaccination (n = 200)	Follow-up visit subset: 3.5 years post-vaccination (n = 165)
Participant age at cohort visit: mean (SD)	6.0 (1.3)	7.4 (1.3)
Participant age during vaccination campaign: mean (SD)	4.0 (1.3)	3.9 (1.3)
Female gender: N (%)	103 (51.5)	86 (52.8)
MenA rSBA: GMT (95% CI)	2870.9 (2517.2 – 3274.4)	1686.1 (1416.0 – 2007.8)
MenA IgG ELISA: GMC (95% CI)	3.8 (3.2 – 4.5)	4.9 (4.1 – 5.8)
Micronutrient status: median (IQR)		
Ferritin (μg/L)	41.4 (23.9 – 63.5)	51.8 (35.6 – 67.5)
Soluble transferrin receptor (mg/L)	7.2 (6.1 – 8.7)	6.5 (5.8 – 7.7)
Retinol binding protein (μmol/L)	0.9 (0.8 – 1.1)	1.0 (0.8 – 1.1)
Micronutrient status: N (%)		
Low ferritin	26 (13.0)	6 (3.7)
High sTfR	60 (30.0)	25 (15.3)
Low RBP	37 (18.5)	37 (22.7)
Anthropometric measurements		
Height-for-age Z-score (HAZ): mean (SD)	-0.7 (1.2)	-0.5 (1.1)
Weight-for-age Z-score (WAZ): mean (SD)	-0.8 (1.1)	-0.8 (1.0)
Inflammation markers: median (IQR)		
C-reactive protein (mg/L)	0.4 (0.2 – 1.2)	0.3 (0.1 – 0.7)
Alpha-1 acid glycoprotein (g/L)	0.8 (0.7 – 1.1)	0.6 (0.5 – 0.8)
Inflammation marker status: N (%)		
High CRP[a]	13 (6.5)	7 (4.3)
High AGP	57 (28.5)	23 (14.2)

[a] One individual was missing information for CRP at the follow-up visit.

Table 7. Linear regression analyses with proportional increase or decrease in antibody titer at 2 years post-vaccination as the outcome.[a]

Covariate	Fold change (adjusted)[b]	95% CI	
rSBA			
Log ferritin	0.40	0.20	0.81
Log sTfR	2.49	0.43	14.32
RBP	1.12	0.47	2.65

[a] Each row in the table represents a separate regression analysis, adjusted for participant age at enrollment, sex, CRP, AGP, HAZ and WAZ.

[b] Coefficients are interpreted as the fold change in rSBA at 2 years post-vaccination per unit change in log ferritin, log sTfR, or RBP.

Table 8. Linear regression analysis with proportional increase or decrease antibody titer difference from the first cohort visit to the second visit as the outcome. [a]

Covariate	Fold change (adjusted)[b]	95% CI	
rSBA			
Log ferritin	1.63	0.78	3.41
Log sTfR	0.41	0.07	2.48
RBP	0.67	0.28	1.61

[a] Each row heading in the table represents a separate regression analysis, adjusted for participant age at enrollment, sex, CRP, AGP, HAZ and WAZ.

[b] Coefficients are interpreted as the fold change in rSBA from 2 to 3.5 years post-vaccination, per unit change in log ferritin, log sTfR, or RBP.

Table 9. Marginal predicted values of rSBA, based on linear regression analyses with antibody titer at 2 years post-vaccination as the outcome.[a]

Covariate	rSBA value (adjusted) [b]	95% CI	
Ferritin			
1st quintile	3246.32	2524.79	4174.04
2nd quintile	2633.31	2195.13	3158.97
3rd quintile	2300.78	1893.40	2795.83
4th quintile	2014.48	1572.58	2580.54
sTfR			
1st quintile	2329.06	1837.06	2952.80
2nd quintile	2427.00	1985.92	2966.06
3rd quintile	2566.63	2135.99	3084.11
4th quintile	2705.84	2194.82	3335.83
RBP			
1st quintile	2585.58	2037.31	3281.39
2nd quintile	2565.08	2107.08	3122.64
3rd quintile	2539.24	2106.03	3061.58
4th quintile	2513.67	1996.85	3164.23

[a] Each row heading in the table represents a separate regression analysis, adjusted for participant age at enrollment, sex, CRP, AGP, HAZ and WAZ.

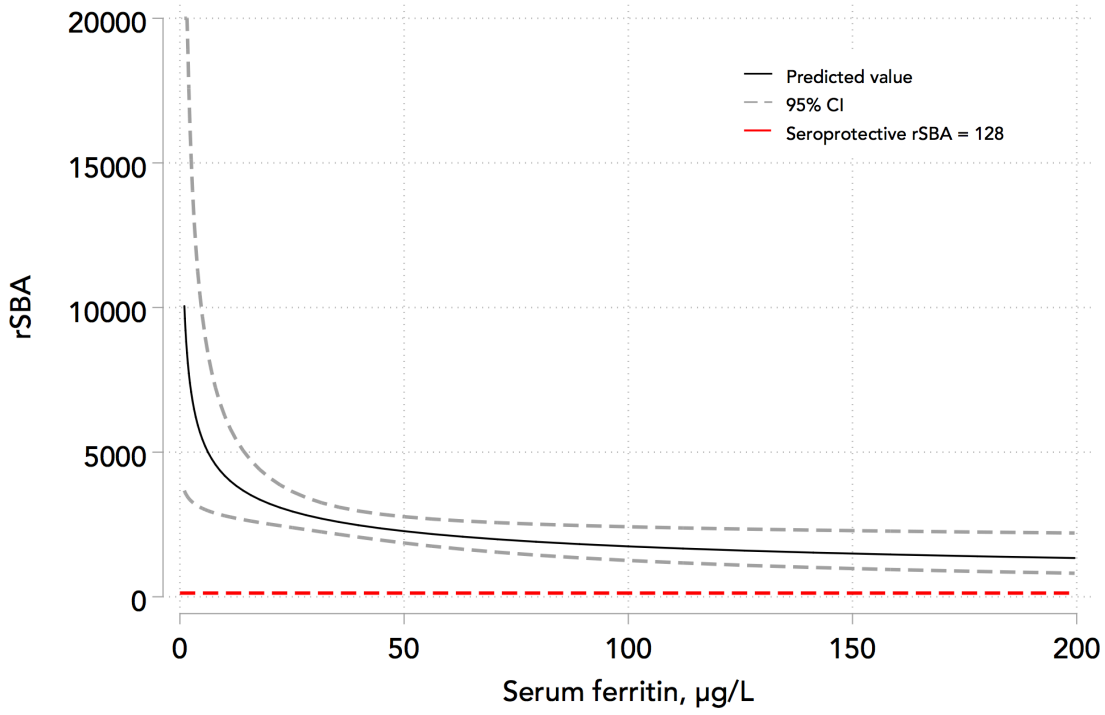
Table 10. Linear regression analysis with proportional increase or decrease in antibody titer at 3.5 years post-vaccination, compared to 2 years post-vaccination.[a]

Covariate	Fold change in rSBA from 2 to 3.5 years post-vaccination (adjusted) [b]	95% CI	
Ferritin			
1st quintile	0.54	0.40	0.73
2nd quintile	0.61	0.49	0.76
3rd quintile	0.66	0.52	0.83
4th quintile	0.71	0.53	0.95
sTfR			
1st quintile	0.67	0.51	0.89
2nd quintile	0.65	0.51	0.82
3rd quintile	0.61	0.49	0.76
4th quintile	0.57	0.44	0.74
RBP			
1st quintile	0.67	0.51	0.89
2nd quintile	0.64	0.51	0.81
3rd quintile	0.60	0.48	0.75
4th quintile	0.56	0.43	0.74

[a] Each row heading in the table represents a separate regression analysis, adjusted for participant age at enrollment, sex, CRP, AGP, HAZ and WAZ.

[b] A fold change of 1 indicates no change in antibody titer; a fold change of 0.5 indicates a 50% decrease in antibody titer at 3.5 years post-vaccination compared to 2 years post-vaccination.

Figure 3. Predicted mean values of rSBA according to serum ferritin at 2 years post-vaccination. [a]



[a] Upper bound values for the 95% confidence interval are cut off at rSBA = 20000 for ease of viewing. The maximum value for the upper bound is 27523.1.

Figure 4. Predicted mean values of rSBA according to sTfR at 2 years post-vaccination.

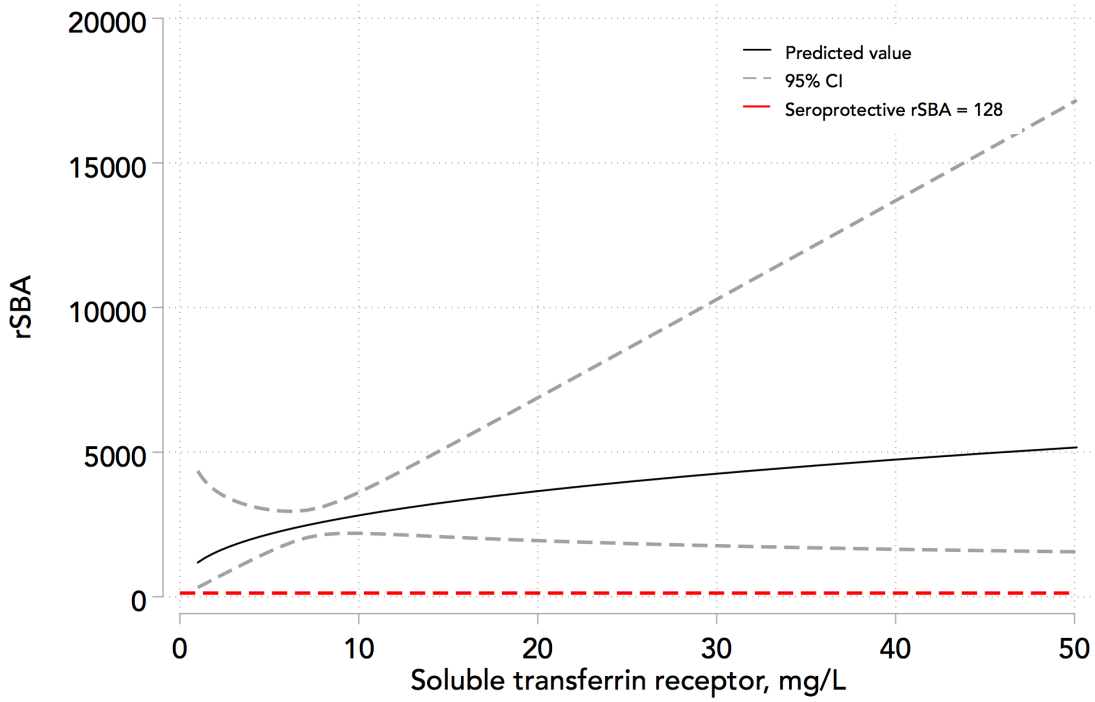


Figure 5. Predicted mean values of rSBA according to RBP at 2 years post-vaccination.

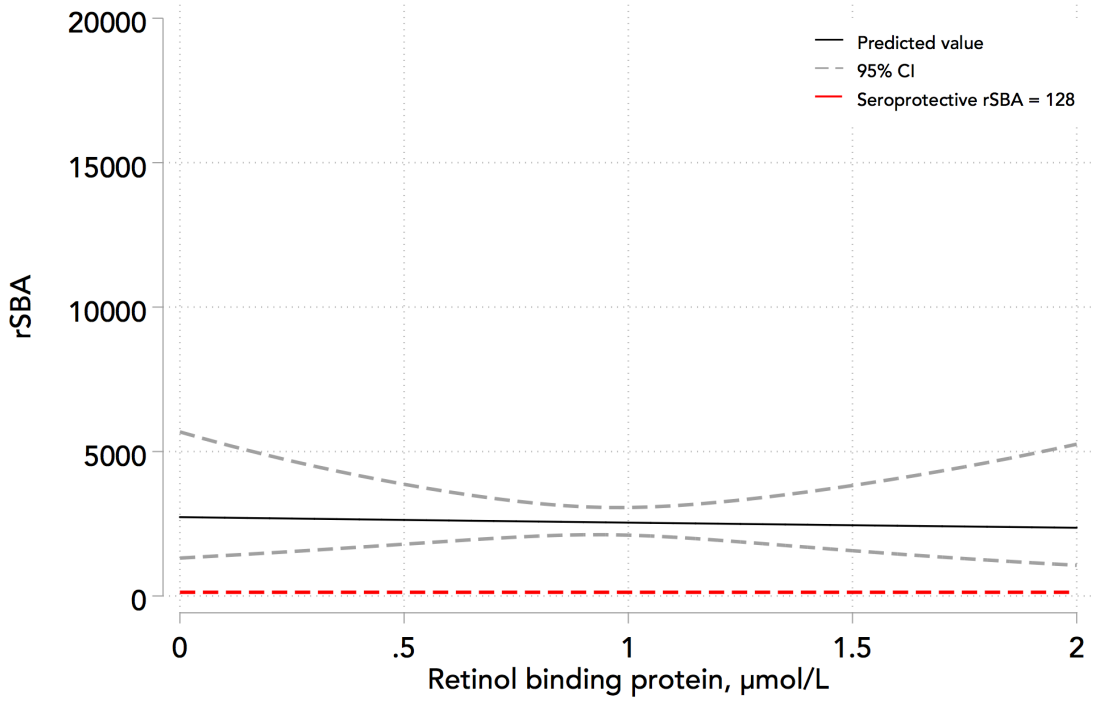


Figure 6. Predicted mean values of the fold change in rSBA from 2 to 3.5 years post-vaccination, according to serum ferritin at 2 years post-vaccination.

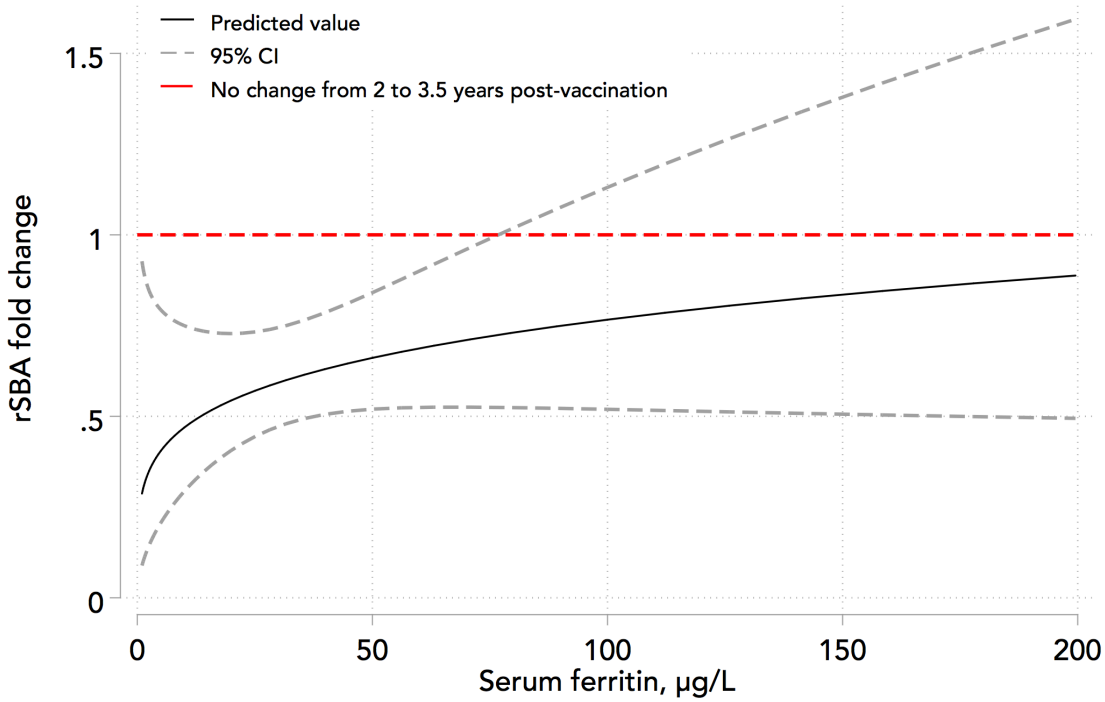


Figure 7. Predicted mean values of the fold change in rSBA from 2 to 3.5 years post-vaccination, according to sTfR at 2 years post-vaccination.

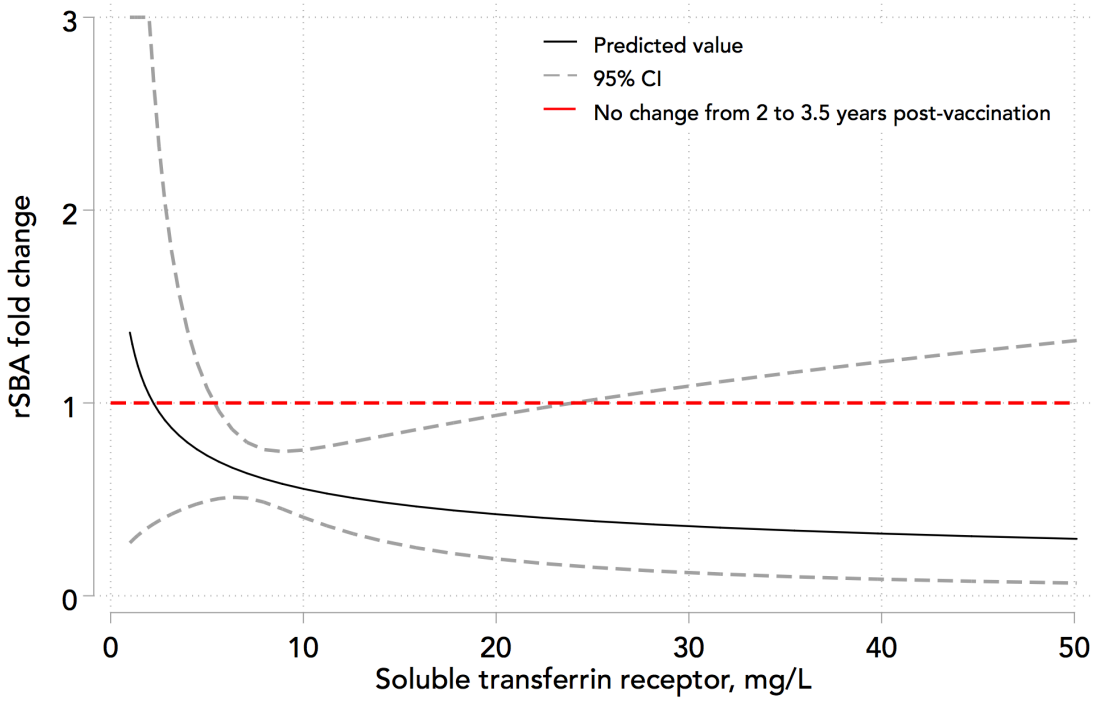
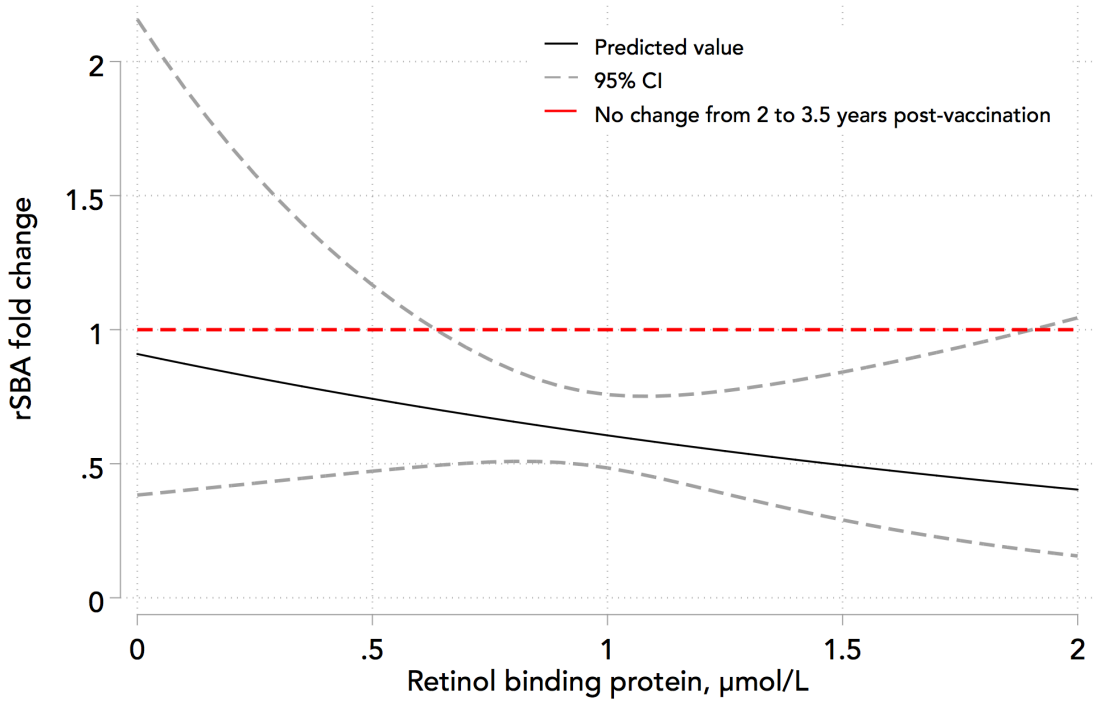


Figure 8. Predicted mean values of the fold change in rSBA from 2 to 3.5 years post-vaccination, according to RBP at 2 years post-vaccination.



Chapter 3: Iron status and nasopharyngeal carriage of *Neisseria meningitidis*

Introduction

Meningococcal meningitis caused by the bacterium *Neisseria meningitidis* (Nm) has historically been a serious public health problem in the African meningitis belt (7, 12). Disease outbreaks due to Nm can be difficult to predict. The prevalence of asymptomatic carriage of Nm is recognized as a predictive factor in meningococcal meningitis outbreaks (43, 44), but it has been noted that relatively little is known about the natural history of nasopharyngeal carriage of meningococcal meningitis (45, 46). A better understanding of the risk factors for Nm carriage could help contextualize the high variation in carriage prevalence and carriage type seen across the meningitis belt (45, 46).

One potential factor affecting risk of Nm carriage is human iron status. Iron is an important component of immune system function and can affect immune function by interacting with T-, B-, and NK-cells (37, 82). Iron deficiency and iron overload have both been implicated in poor immune function (35, 37). Low iron has been associated with reduced cell-mediated immune function (83) and reduced cytotoxic activity against pathogens (83, 84), but several studies have noted that this latter association may be confounded in observational studies by other demographic factors (83), including socio-economic status (85).

Conversely, high iron is associated with increased risk of infection (35, 37, 67), especially for malaria (70, 86). The immune response to infection can also affect iron levels: during infection, the body sequesters iron in order to avoid the inactivation of macrophages and their subsequent immune effector pathways (37).

In addition to existing host interactions between immune function and iron metabolism, most pathogens—including Nm—require iron in order to replicate and have therefore evolved specialized mechanisms for obtaining iron from human hosts (87). Given these iron-immunity and host-pathogen interactions, it is therefore possible that individual iron status may be related to the risk for successful Nm colonization. We hypothesized that asymptomatic nasopharyngeal Nm carriage among children and adults in the African meningitis belt would differ based on iron status [measured by serum ferritin and soluble transferrin receptor (sTfR)]. We investigated this hypothesis using a matched case-control study design applied to a large-scale cohort study on meningococcal carriage in the African meningitis belt.

Methods

Setting and study participants

This is a retrospective case-control study nested within a cohort study originally conducted by the African Meningococcal Carriage Consortium (MenAfriCar Consortium), a partnership between seven African and twelve

northern research institutes (37). The primary aim of the Consortium is to investigate the epidemiology of nasopharyngeal carriage of Nm in the African meningitis belt (88). The methods used by the Consortium to design and implement longitudinal cohort studies have been described in detail elsewhere (36). This analysis was completed using data collected from MenAfriCar Consortium pre-vaccination cross-sectional surveys and household contact surveys, conducted in seven countries in the meningitis belt (Chad, Ethiopia, Ghana, Mali, Niger, Nigeria, and Senegal) during 2010-2012, according to a common protocol (45, 88, 89). In each country, participants were drawn from one rural and one urban study site; eligible households in each study area were then identified using simple random sampling from a current demographic surveillance system or a census conducted specifically for the study. Within households, individuals were selected by age-stratified random sampling to be invited to participate in the original study (age groups defined as < 1 year, 1-4 years, 5-14 years, 15-29 years, and ≥ 30 years). Three cross-sectional surveys were then conducted at each site using this design: two in rainy seasons and one in a dry season. Participants who consented to participate in the study provided demographic and household information through a structured questionnaire. Study staff collected a nasopharyngeal swab (that touched both the posterior pharynx and the tonsils). In addition, study staff randomly selected an age-stratified random sample of 400 individuals ≥ 6 months of age in each study from the larger sample and collected a 5mL blood sample to be tested for the

presence of antibody to Nm. For our secondary analysis, these blood samples were also tested for nutritional biomarkers including serum ferritin and sTfR.

Identification of an individual testing positive for Nm at the initial cross-sectional visit triggered a second visit to the household, during which all household members were invited to participate in a follow-up, longitudinal household study. In the longitudinal study, consenting household members provided nasopharyngeal swabs twice a month for 2 months and then monthly for an additional 4 months, as well as a 5mL blood sample at the first visit (88). In these studies, an age-stratified sample size of 800 individuals per research center was calculated to be the number of individuals required to allow meaningful comparisons across centers (90). These studies were approved by the London School of Hygiene and Tropical Medicine Ethics Committee as well as the ethics committees of each of the seven African partner institutions, except for Chad, which did not have a formal ethical committee. In Chad, approval was granted by a special Ministry of Health committee established to oversee the studies (88).

We used a case-control study design to analyze the relationship between iron status and risk of Nm carriage in these study participants. We defined cases as individuals who participated in either the cross-sectional or the longitudinal Nm carriage study visits, whose nasopharyngeal samples tested positive for Nm and who provided a blood sample for testing at the time they provided a nasopharyngeal sample. Controls were participants in cross-sectional or

longitudinal visits whose nasopharyngeal samples tested negative for Nm and who provided a blood sample for testing at the same time they provided a nasopharyngeal sample. Cases were matched to up to two controls based on age (range: plus or minus 1 year), sex, study site, and date of swab (range: plus or minus 30 days). Since baseline iron status (75) and risk of Nm carriage (3) can both differ by age, we conducted this analysis stratified by age groups (6 months-4 years, 5-11 years, 12-17 years, and ≥ 18 years old).

Laboratory methods

The precise laboratory methods for assessment of Nm carriage (88) and nutritional biomarkers (45, 88, 89) have been described in detail elsewhere. Briefly, study staff collected pharyngeal swabs from participants and plated the swabs directly onto modified Thayer-Martin agar plates in the field. One suspected colony with morphology indicative of *Neisseria* species was cultured onto two blood agar plates, which were then incubated at 37° C for 18-24 hours. Cultured bacteria were then tested for oxidase activity and Gram stained. Bacteria that were oxidase-positive and Gram-negative were tested for γ-glutamyl-transferase activity (GGT), β-galactosidase activity with ortho-nitrophenyl-β-D-galactopyranoside (ONPG), and butyrate esterase activity (Tributyrin). Isolates that were GGT positive, ONPG negative and Tributyrin negative were identified as Nm species and serogrouped by slide agglutination. To confirm the result of *Neisseria* spp. identified using culture techniques, the

DNA of oxidase-positive, Gram-negative bacteria were amplified and sequenced in a hierarchical analysis with a high-throughput Sanger sequencing pipeline (88). Blood serum samples were also tested for serum ferritin and sTfR (nutritional biomarkers representing iron status), and C-reactive protein (CRP, an acute phase protein) using a multiplex sandwich ELISA with HRP-coupled antibodies (88). In the absence of infection, ferritin reflects dietary iron status. However, serum ferritin is an acute phase protein, meaning its blood concentrations increase during acute infection, regardless of dietary iron status. Therefore, we used CRP—another acute phase protein—to determine acute infection status and aid interpretation of serum ferritin levels. sTfR levels are less affected by acute infection (74) and reflect availability of iron in the bone marrow.

Statistical analysis

We used conditional logistic regression models to assess whether there was an association between serum ferritin or sTfR and risk of Nm carriage, using continuous $\log_{10}$ ferritin and continuous $\log_{10}$ sTfR as independent variables. We $\log_{10}$-transformed ferritin and sTfR for these analyses because the underlying distributions of both biomarkers were heavily right-skewed. Since cases and controls were matched on age, sex, study site, and date of swab, these covariates were not included in any of the adjusted regression models. However, we controlled for the number of residents in the household, and ran separate regression models excluding individuals likely to have acute infection (CRP > 5

mg/L). Additionally, since baseline iron status and risk of carriage can both vary by age, we report age-stratified results (children < 5, children 5-11, children 12-17, and adults ≥ 18 years old). All analyses were performed using Stata 15 (College Station, TX).

Results

We identified 194 cases nested within the larger study who met the eligibility criteria and who were matched on age, sex, site, and swab date to 332 controls also drawn from the larger study (Figure 1). Of the 194 cases, 138 (71.1%) were matched to two controls; the remaining 56 cases were matched to one control. There were 281 (53.4%) female participants; 329 (62.6%) were children under the age of 18 (**Table 11**). The median (IQR) serum ferritin was 51.2 μg/L (29.5 – 89.4 μg/L); the median (IQR) sTfR was 7.4 (5.8 – 11.8) mg/L (**Table 11**). Participants reported a median number of 6 (IQR: 5 – 9) residents living in their household (**Table 11**).

In an unadjusted conditional logistic regression, increasing log serum ferritin was significantly related to decreased odds of Nm carriage in children 5-11 years old (uOR: 0.57, 95% CI: 0.34 – 0.98). After adjusting for the number of residents in the household, results were similar (aOR: 0.28, 95% CI: 0.11 – 0.68) (**Table 12**). In a sensitivity analysis excluding individuals with CRP > 5 mg/L, this relationship remained (aOR: 0.30, 95% CI: 0.12 – 0.78), though it was statistically significant in the adjusted regression model only (**Table 12**).

Conditional logistic regressions did not identify a relationship between log serum ferritin and carriage for children less than 5 years old, children 12-17 years old, or adults ≥ 18 years old; excluding individuals with CRP > 5 mg/L yielded similar results (**Table 12**).

In unadjusted conditional logistic regressions, increasing sTfR was significantly related to decreased odds of Nm carriage in children 12-17 years old (**Table 13**); these results were similar including or excluding individuals with CRP > 5 mg/L. However, this relationship did not exist in any other age group, and the effect did not remain after adjusting for the number of residents in the household (**Table 13**).

Discussion

In our analysis, increasing log serum ferritin was significantly related to reduced odds of Nm carriage in children 5-11 years old. These results remained after excluding individuals with CRP > 5 mg/L (those likely to have acute infection); however, similar results were not seen in other age groups. Similarly, sTfR was significantly related to reduced odds of Nm carriage in children 12-17 years old, though these results did not remain after adjusting for the number of residents in the household. Though past studies have considered other demographic and social characteristics related to the risk of Nm carriage, including age and sex (75), household crowding and kitchen location (87), rainy vs. dry season (91), risky behaviors such as smoking and kissing (91), and co-

colonization with other, non-pathogenic *Neisseria* species (92), relatively few studies have investigated individual biological characteristics associated with Nm carriage (91). Despite the established relationship between iron and immune system function, this study is the first to investigate whether iron biomarkers are related to the odds of Nm carriage.

Since ferritin is an acute phase iron storage protein, higher levels of ferritin can either indicate higher dietary iron or a higher degree of iron sequestration during acute infection (35). Our analysis finds similar results when individuals with CRP > 5 mg/L are excluded, so it is likely that higher ferritin in this group of individuals is indicative of dietary iron status rather than a result of acute infection. Additionally, our analysis identified a statistically significant relationship between increasing sTfR and reduced odds of Nm carriage among 12-17 year-olds; since sTfR is less apt to change during acute infection, it is likely that our findings reflect the relationship between dietary iron and Nm carriage risk.

This analysis has three main limitations. First, although this analysis utilized all available cases from a larger carriage study, and the majority of cases were matched to two controls, there still remained a moderately high level of variability in estimates obtained from conditional logistic regression models. Since point estimates with and without adjustment were similar, and point estimates including and excluding individuals with high CRP are similar, it is plausible that a larger sample size would reveal similar point estimates with smaller 95% confidence intervals. However, given the information currently

available from this analysis, we cannot conclude whether this relationship would be consistent given a larger sample size.

Second, information about iron status was only collected at one time point during carriage (at one time point only for controls), and this analysis identified carriage status at only one time point. Therefore, our analysis cannot identify whether high iron occurs before or after nasopharyngeal Nm colonization. However, it is unlikely that nasopharyngeal carriage would effect subsequent changes iron status through inflammation, as it has been noted that nasopharyngeal carriage does not activate an acute inflammatory immune response (93).

Finally, this analysis measured iron using serum ferritin and sTfR as biomarkers. However, there are many other biomarkers of iron that are capable of describing different aspects of iron's role in immune function and acute inflammation. For example, it is known that Nm uses human lactoferrin for its iron needs (94), and that lactoferrin is more highly active at mucosal surfaces (as compared to serum ferritin and sTfR, which are systemically active) (95). Incorporating information from these additional iron biomarkers in future analyses may therefore provide a more complete picture of the potential relationship between iron and asymptomatic Nm carriage.

Conclusions

Our analysis identified a relationship between increasing log serum ferritin and reduced odds of Nm carriage in children 5-11 years old, and increasing sTfR and reduced odds of Nm carriage in children 12-17 years old. These relationships remained after excluding individuals with CRP > 5 mg/L, but were not seen in other age groups. Future studies should focus on high-risk populations for carriage, including children, and consider other iron biomarkers which may interact directly with Nm, including lactoferrin.

Table 11. Descriptive and anthropometric characteristics of study participants.

Characteristics	Controls N = 332	Cases N = 194
Female sex: N (%)	178 (53.6)	103 (53.1)
Country: N (%)		
Mali	32 (9.6)	17 (8.8)
Niger	221 (66.6)	131 (67.5)
Ghana	2 (0.6)	2 (1.0)
Nigeria	1 (0.3)	1 (0.5)
Senegal	16 (4.8)	11 (5.7)
Ethiopia	60 (18.1)	32 (16.5)
Meningitis vaccination		
No	227 (68.4)	131 (67.5)
In past year	83 (25.0)	49 (25.3)
In past 3 years	22 (6.6)	12 (6.2)
Don't know	0 (0)	2 (1.0)
Age group		
6 months-5 years	33 (9.9)	18 (9.3)
5-11 years	95 (28.6)	54 (27.8)
12-17 years	81 (24.4)	48 (24.7)
≥ 18 years	123 (37.1)	74 (38.1)
Number of residents in the household	6 (4 – 9)	6 (5 – 9)
Iron biomarkers: median (IQR)		
Serum ferritin (μg/L)	52.7 (31.7 – 94.6)	47.8 (25.7 – 80.8)
sTfR (mg/L)	7.3 (5.8 – 11.6)	7.4 (5.8 – 12.3)
Inflammatory marker: median (IQR)		
CRP (mg/L)	0.8 (0.3 – 2.9)	0.8 (0.3 – 2.2)

Table 12. Conditional logistic regressions describing the odds of carriage according to log serum ferritin, by age group and CRP levels.[a]

Characteristic	uOR	95% CI	aOR[b]	95% CI
Age-stratified models: all individuals				
Children < 5 years	1.24	0.55 – 2.80	1.33	0.54 – 3.26
Children 5-11 years	0.57	0.34 – 0.98	0.28	0.11 – 0.68
Children 12-17 years	0.77	0.47 – 1.28	0.73	0.43 – 1.25
Adults ≥ 18 years	0.78	0.50 – 1.20	0.87	0.54 – 1.41
Age-stratified models: including only individuals with CRP < 5 mg/L				
Children < 5 years	0.92	0.26 – 3.27	1.20	0.26 – 5.48
Children 5-11 years	0.60	0.31 – 1.15	0.30	0.12 – 0.78
Children 12-17 years	0.77	0.43 – 1.39	0.70	0.37 – 1.33
Adults ≥ 18 years	0.91	0.55 – 1.51	0.97	0.56 – 1.67

[a] Each row represents results from one conditional logistic regression, where cases and controls were matched on age, sex, study site, and date of swab.

[b] Adjusted models control for number of residents in the household.

Table 13. Conditional logistic regressions describing the odds of carriage according to log soluble transferrin receptor, by age group and CRP levels. [a]

Characteristic	uOR	95% CI	aOR[b]	95% CI
Age-stratified models: all individuals				
Children < 5	0.76	0.21 – 2.74	0.74	0.19 – 2.88
Children 5-11 years	1.10	0.49 – 2.44	1.62	0.58 – 4.53
Children 12-17 years	0.34	0.12 – 0.98	0.42	0.15 – 1.24
Adults ≥ 18	1.25	0.58 – 2.71	0.96	0.42 – 2.21
Age-stratified models: including only individuals with CRP < 5 mg/L				
Children < 5	0.81	0.15 – 4.30	0.74	0.11, 5.20
Children 5-11 years	1.11	0.43 – 2.89	2.01	0.62 – 6.62
Children 12-17 years	0.31	0.10 – 0.96	0.36	0.11 – 1.15
Adults ≥ 18	1.96	0.76 – 5.09	1.53	0.56, 4.12

[a] Each row represents results from one conditional logistic regression, where cases and controls were matched on age, sex, study site, and date of swab.

[b] Adjusted models control for number of residents in the household.

Conclusions

Main findings

This dissertation investigated three potential relationships between nutrition and immune function in the context of meningococcal disease and vaccine response in the African meningitis belt. In children 5 years old and younger, anthropometric Z-scores for height-for-age (HAZ), weight-for-height (WHZ), and weight-for-age (WAZ) were not significantly associated with pre-vaccination antibody titers; nor were they related to the change in antibody from pre-vaccination to 28-35 days post vaccination with either a polysaccharide or a polysaccharide-protein conjugate vaccine. Although there was a significant decline in vaccine-elicited antibody over time during the year post-vaccination, HAZ and WHZ were not found to be related to this decline. However, higher WAZ was significantly associated with an increase in antibody elicited by polysaccharide vaccine in the year post-vaccination.

In a different group of children 9 years old and younger, increasing serum ferritin was associated with a decrease in antibody elicited by polysaccharide-protein conjugate vaccine at both 2 and 3.5 years post-vaccination (though this relationship was only statistically significant at 2 years post-vaccination). However, soluble transferrin receptor (sTfR) and retinol binding protein (RBP) were both unrelated to levels of vaccine-elicited antibody at 2 and 3.5 years post-

vaccination. Neither serum ferritin, sTfR, nor RBP were related to the fold change in vaccine-elicited antibody from 2 to 3.5 years post-vaccination.

Finally, in a third population of children and adults, both increasing serum ferritin and increasing sTfR were related to reduced odds of asymptomatic nasopharyngeal carriage of *Neisseria meningitidis*. Although these results were only seen for specific age groups, the findings remained after excluding individuals with likely concurrent acute infection.

Strengths and limitations

Each analysis in this dissertation makes novel use of existing data to answer questions regarding nutrition and immune function that are directly applicable to individuals living in the African meningitis belt. Since PEU, iron deficiency, and vitamin A deficiency are relatively common in the African meningitis belt, identifying the relationships between immune function and nutrition in this area can provide additional insight into the risks surrounding meningococcal disease. Although many studies have already noted relationships between undernutrition and other infectious diseases including respiratory diseases and diarrhea (29, 63), measles (96), poliovirus (27), and malaria (70, 86), no study has yet assessed the relationship between nutrition and immune function in the context of meningococcal disease.

Each analysis in this dissertation is a secondary analysis of data originally collected for a different purpose. As such, these analyses are subject to the

dangers of secondary data use, including potential unmeasured confounding and study design limitations. Additionally, these studies did not seek specifically to enroll individuals with PEU or deficiency in iron or vitamin A, and in some cases may have directly excluded individuals with severe nutritional deficits. As a result, although the results from these studies are likely to be more broadly generalizable to individuals living in the African meningitis belt, they may not identify relationships between nutrition and immune function seen in individuals with severe nutritional deficits.

Implications

Existing research on the immunogenicity, efficacy, and effectiveness of meningococcal vaccines in areas with high levels of undernutrition can benefit from the results of this dissertation. A confirmation that PEU is not related to vaccine immunogenicity and is not consistently related to the decline in vaccine-elicited antibody over a year post-vaccination means that meningococcal vaccines can be used to help disrupt the “vicious cycle” of infection and malnutrition, as it is an intervention that is effective in children regardless of protein-energy nutritional status. It is additionally reassuring that these analyses found no relationship between RBP and meningococcal vaccine-elicited antibody, since vitamin A deficiency has long been implicated in poor immune function and is considered to be a major component of the “vicious cycle” of infection and malnutrition (97).

However, the findings that increasing serum ferritin in children may be related to reduced long-term persistence of vaccine-elicited antibody, as well as reduced odds of *Neisseria meningitidis* carriage, warrant further research on this topic. Taken together, these findings suggest a potential scenario in which children with higher serum ferritin are less likely to be exposed to Nm carriage, and may therefore lose a potential prime-boost effect in antibody after vaccination, thus comparatively reducing the level of vaccine-elicited antibody at longer time periods post-vaccination. However, since neither of the original studies that supported these analyses were designed to answer this question, and others have noted the potential for unmeasured confounding in studies on iron and immune function (83, 85). such a conclusion cannot be made from these findings alone. Further research is needed to identify potential direct relationships between serum ferritin and immune function in the context of meningococcal disease.

Future research

This dissertation represents the first time that iron status and meningococcal disease have been studied with an epidemiological approach. As mentioned above, further research is needed to identify potential direct relationships between serum ferritin and immune function in the context of the immune response to meningococcal vaccination and meningococcal disease. The analyses in this dissertation that identified a link between iron status and

immune response to meningococcal disease and meningococcal vaccines were not originally designed to answer this question, and as such cannot identify any potential direction of causality. These studies cannot identify whether pre-vaccination iron status is related to long-term antibody persistence, or whether iron status over time is related to long-term antibody persistence Similarly, these studies cannot identify whether high iron effects change the odds of Nm carriage, or whether Nm carriage affects iron status. In order to elucidate these findings, longitudinal study designs are required, which would assess iron status and either Nm carriage or vaccine-elicited antibody at several time points pre- and post-vaccination. Additionally, as previously mentioned, there exist several different biomarkers of iron status, each of which could have a unique relationship with the immune response to meningococcal vaccination or Nm carriage. For example, Nm is known to scavenge iron from human lactoferrin in the nasopharynx, and it is believed that lactoferrin is the primary iron source for Nm (94). Therefore, future studies should consider additional biomarkers of iron, including specifically lactoferrin, to identify potential direct relationships between iron status and the immune response to meningococcal vaccination and meningococcal disease.

Summary and contribution

These analyses do not find consistent evidence of a relationship between PEU and meningococcal vaccine immunogenicity, and find no evidence of a

relationship between RBP and vaccine-elicited antibody persistence. However, the findings of these analyses identify a potential relationship between iron status and vaccine-elicited antibody persistence as well as the odds of Nm carriage. These findings contribute to the robust existing literature surrounding immune response to meningococcal vaccination and factors associated with meningococcal disease. They also complement the robust literature investigating potential relationships between undernutrition and infectious disease risk and vaccine immunogenicity.

References

1. Greenwood B. Meningococcal meningitis in Africa. Trans R Soc Trop Med Hyg. 1999;93:341-53.

2. Meningitis control in countries of the African meningitis belt, 2015. Geneva, Switzerland: World Health Organization, 2016.

3. Stephens DS, Greenwood B, Brandtzaeg P. Epidemic meningitis, meningococcaemia, and Neisseria meningitidis. The Lancet. 2007;369(9580):2196-210.

4. Meningococcal: Causes and transmission Atlanta, GA: Centers for Disease Control and Prevention; 2015. Available from: https://www.cdc.gov/meningococcal/about/causes-transmission.html.

5. Meningococal meningitis Geneva, Switzerland: World Health Organization; 2015. Available from: http://www.who.int/mediacentre/factsheets/fs141/en/.

6. Meningococcal Disease in Other Countries Atlanta, GA2016. Available from: http://www.cdc.gov/meningococcal/global.html.

7. Jafri RZ, Ali A, Messonnier N, Tevi-Benissan C, Durrheim D, Eskola J, et al. Global epidemiology of invasive meningococcal disease. Population Health Metrics. 2013;11(17):1-9.

8. Meningococcal disease: situation in the African Meningitis Belt Geneva, Switzerland2012. Available from: http://www.who.int/csr/don/2012_03_23/en/.

9. Deuren Mv, Brandzaeg P, Meer JWMvd. Update on meningococcal disease with emphasis on pathogenesis and clinical management. Clin Microbiol Rev. 2000;13(1):144-66.

10. Apicella M, Baart G, Baumann C, Biais N, Bos M, Brandtzaeg P, et al. Neisseria meningitidis: Advanced methods and protocols. 1 ed. Hatfield, Hertfordshire, United Kingdom: Springer; 2012.

11. Doran KS, Fulde M, Gratz N, Kim BJ, Nau R, Prasadarao N, et al. Host-pathogen interactions in bacterial meningitis. Acta Neuropathol. 2016;131(2):185-209.

12. Meningococcal disease in countries of the African meningitis belt, 2012 - emerging needs and future perspectives. Wkly Epidemiol Rec. 2013;12(88):129-36.

13. Lapeyssonnie L. La méningite cérébro-spinale en Afrique. Bull World Health Organ. 1963;28:Suppl. 1-114.

14. LaForce FM, Konde K, Viviani S, Preziosi MP. The Meningitis Vaccine Project. Vaccine. 2007;25 Suppl 1:A97-100.

15. Control of epidemic meningococcal disease. WHO practical guidelines. 2nd edition. Geneva, Switzerland: World Health Organization, 1998.

16. Meningococcal disease in countries of the African meningitis belt, 2012 -- emerging needs and future perspectives. Wkly Epidemiol Rec. 2013;12(88):129-36.

17. Aguado MT, Jodar L, Granoff D, Rabinovich R, Ceccarini C, Perkin GW. From Epidemic Meningitis Vaccines for Africa to the Meningitis Vaccine Project. Clin Infect Dis. 2015;61 Suppl 5:S391-5.

18. Meningococcal disease Geneva, Switzerland: World Health Organization; 2012. Available from: http://www.who.int/ith/vaccines/meningococcal/en/.

19. Djingarey MH, Diomande FV, Barry R, Kandolo D, Shirehwa F, Lingani C, et al. Introduction and Rollout of a New Group A Meningococcal Conjugate Vaccine (PsA-TT) in African Meningitis Belt Countries, 2010-2014. Clin Infect Dis. 2015;61 Suppl 5:S434-41.

20. Tapia MD, Sow SO, Haidara FC, Diallo F, Doumbia M, Enwere GC, et al. A phase 3, double-blind, randomized, active controlled study to evaluate the safety of MenAfriVac in healthy Malians. Clin Infect Dis. 2015;61 Suppl 5:S507-13.

21. Price GA, Hollander AM, Plikaytis BD, Mocca BT, Carlone G, Findlow H, et al. Human complement bactericidal responses to a group A meningococcal conjugate vaccine in Africans and comparison to responses measured by 2 other group A immunoassays. Clin Infect Dis. 2015;61 Suppl 5:S554-62.

22. Basta NE, Borrow R, Berthe A, Dembele AT, Onwuchekwa U, Townsend K, et al. Population-level persistence of immunity 2 years after the PsA-TT mass-vaccination campaign in Mali. Clin Infect Dis. 2015;61 Suppl 5:S547-53.

23. Tall H, Yaro S, Kpoda HB, Ouangraoua S, Trotter CL, Njanpop Lafourcade BM, et al. Meningococcal Seroepidemiology 1 Year After the PsA-TT

Mass Immunization Campaign in Burkina Faso. Clin Infect Dis. 2015;61 Suppl 5:S540-6.

24. CDC Global Health - Stories - An update on global partners reaching the ultimate goal of eliminating epidemic meningitis as a public health concern in Africa Atlanta, GA: Centers for Disease Control and Prevention; 2015 [updated April 23, 2015]. Available from: http://www.cdc.gov/globalhealth/stories/meningitis.htm.

25. Tang Y, Plikaytis BD, Preziosi MP, Borrow R. Influence of age on antibody response and persistence following immunization with MenAfriVac. Clin Infect Dis. 2015;61 Suppl 5:S531-9.

26. Idoko OT, Okolo SN, Plikaytis B, Akinsola A, Viviani S, Borrow R, et al. The impact of pre-existing antibody on subsequent immune responses to meningococcal A-containing vaccines. Vaccine. 2014;32(33):4220-7.

27. Savy M, Edmond K, Fine PE, Hall A, Hennig BJ, Moore SE, et al. Landscape analysis of interactions between nutrition and vaccine responses in children. J Nutr. 2009;139(11):2154S-218S.

28. Prendergast AJ. Malnutrition and vaccination in developing countries. Philos Trans R Soc Lond B Biol Sci. 2015;370(1671).

29. Scrimshaw NS, SanGiovanni JP. Synergism of nutrition, infection, and immunity: an overview. Am J Clin Nutr. 1997;66:464S-77S.

30. Olofin I, McDonald CM, Ezzati M, Flaxman S, Black RE, Fawzi WW, et al. Associations of suboptimal growth with all-cause and cause-specific mortality in

children under five years: a pooled analysis of ten prospective studies. PLoS ONE. 2013;8(5):e64636.

31. Bhutta ZA, Salam RA. Global nutrition epidemiology and trends. Ann Nutr Metab. 2012;61 Suppl 1:19-27.

32. Global prevalence of vitamin A deficiency in populations at risk: 1995-2005. Geneva, Switzerland: World Health Organization, 2009.

33. Worldwide prevalence of anaemia, 1993-2005: WHO global database on anaemia. Geneva, Switzerland: World Health Organization & US Centers for Disease Control and Prevention, 2009.

34. Brown CC, Noelle RJ. Seeing through the dark: New insights into the immune regulatory functions of vitamin A. Eur J Immunol. 2015;45(5):1287-95.

35. Prentice AM. Iron metabolism, malaria, and other infections: what is all the fuss about? J Nutr. 2008;138(12):2537-41.

36. Weinberg ED. Nutritional immunity: host's attempt to withhold iron from microbial invaders. Journal of the American Medical Association. 1975;231(1):39-41.

37. Weiss G. Iron and immunity: a double-edged sword. Eur J Clin Invest. 2002;32 (Suppl. 1):70-8.

38. Weiss G, Wachter H, Fuchs D. Linkage of cell-mediated immunity to iron metabolism. Immunol Today. 1995;16(10):495-500.

39. Schaible UE, Kaufmann SH. Iron and microbial infection. Nat Rev Microbiol. 2004;2(12):946-53.

40. Moya-Alvarez V, Bodeau-Livinec F, Cot M. Iron and malaria: a dangerous liaison? Nutr Rev. 2016;74(10):612-23.

41. Meningococcal Disease: Methods and Protocols. Walker JM, editor. Totowa, New Jersey: Humana Press.

42. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. The Lancet Global Health. 2013;1:e16-25.

43. Stephens DS. Biology and pathogenesis of the evolutionarily successful, obligate human bacterium Neisseria meningitidis. Vaccine. 2009;27 Suppl 2:B71-7.

44. Phair JJ, Schoenbach EB, Root CM. Meningococcal carrier studies. Am J Public Health Nations Health. 1944;34:148-54.

45. MenAfriCar c. The Diversity of Meningococcal Carriage Across the African Meningitis Belt and the Impact of Vaccination With a Group A Meningococcal Conjugate Vaccine. J Infect Dis. 2015;212(8):1298-307.

46. Trotter CL, Greenwood BM. Meningococcal carriage in the African meningitis belt. The Lancet Infectious Diseases. 2007;7(12):797-803.

47. Meningococcal A conjugate vaccine: updated guidance, February 2015. Wkly Epidemiol Rec. 2015;8(90):57-68.

48. Mali: Child malnutrition estimates by WHO Child Growth Standards [Internet]. World Health Organization. 2006 [cited 20 July 2016]. Available from: http://www.who.int/nutgrowthdb/database/countries/who_standards/mli.pdf?ua=1.

49. Senegal: Child malnutrition estimates by WHO Child Growth Standards [Internet]. World Health Organization. 2012 [cited 20 July 2016]. Available from: http://www.who.int/nutgrowthdb/database/countries/who_standards/sen_dat.pdf?ua=1.

50. Gambia: Child malnutrition estimates by WHO Child Growth Standards [Internet]. World Health Organization. 2010 [cited 20 July 2016]. Available from: http://www.who.int/nutgrowthdb/database/countries/who_standards/gmb_dat.pdf?ua=1.

51. Ghana: Child malnutrition estimates by WHO Child Growth Standards [Internet]. World Health Organization. 2011 [cited 20 July 2016]. Available from: http://www.who.int/nutgrowthdb/database/countries/who_standards/gha_dat.pdf?ua=1.

52. Dossetor J, Whittle HC, Greenwood BM. Persistent measles infection in malnourished children. Br Med J. 1977;1:1633-5.

53. Mohammed I, Damisah MM. The immunological response to polyvalent meningococcal vaccine in Bauchi State, Nigeria. Trans R Soc Trop Med Hyg. 1982;76(3):351-3.

54. Greenwood BM, Bradley AK, Blakebrough IS, Whittle HC, Marshall TF, Gilles HM. The immune response to a meningococcal polysaccharide vaccine in an African village. Trans R Soc Trop Med Hyg. 1980;74:340-6.

55. Greenwood BM, Bradley-Moore AM, Bradley AK, Kirkwood BR, Gilles HM. The immune response to vaccination in undernourished and well-nourished Nigerian children. Ann Trop Med Parasitol. 1986;80(5):537-44.

56. Schneerson R, Barrera O, Sutton A, Robbins JB. Preparation, characterization, and immunogenicity of *Haemophilus influenzae* type b polysaccharide-protein conjugates. J Exp Med. 1980;152:361-76.

57. Sow S, Okoko BJ, Diallo A, Viviani S, Borrow R, Carlone G, et al. Immunogenicity and safety of a meningococcal A conjugate vaccine in Africans. N Engl J Med. 2011;364:2293-304.

58. Roy Chowdhury P, Meier C, Laraway H, Tang Y, Hodgson A, Sow SO, et al. Immunogenicity of Yellow Fever Vaccine Coadministered With MenAfriVac in Healthy Infants in Ghana and Mali. Clin Infect Dis. 2015;61 Suppl 5:S586-93.

59. WHO Child Growth Standards. Geneva, Switzerland: World Health Organization, 2006.

60. Enquête Démographique et de Santé (EDSM V), 2012-2013. Bamako, Mali: , Cellule de Planification et de Statistiques (CPS/SSDSPF)

, Institut National de la Statistique (INSTAT)

, Centre d'Études et d'Information Statistiques (INFO-STAT), 2014.

61. Mali: National Anemia Profile. USAID

SPRING, 2015.

62. Schémann J, Banou A, Guindo A, Joret V, Traore L, Malvy D. Prevalence of undernutrition and vitamin A deficiency in the Dogon Region, Mali. J Am Coll Nutr. 2002;21(5):381-7.

63. Sommer A, Djunaedi E, Loeden AA, Tarwotjo I, Keith P. West J, Tilden R. Impact of vitamin A supplementation on childhood mortality. The Lancet. 1986;327(8491):1169-73.

64. Mayo-Wilson E, Imdad A, Herzer K, Yakoob MY, Bhutta ZA. Vitamin A supplements for preventing mortality, illness, and blindness in children aged under 5: systematic review and meta-analysis. BMJ. 2011;343:d5094.

65. Semba R, Muhilal, Scott A, Natadisastra G, Wirasasmita S, Mele L, et al. Depressed immune response to tetanus in children with vitamin A deficiency. J Nutr. 1992;122:101-7.

66. Brüssow H, Sidoti J, Dirren H, Freire WB. Effect of malnutrition in Ecuadorian children on titers of serum antibodies to various microbial agents. Clin Diagn Lab Immunol. 1995;2(1):62-8.

67. Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, et al. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. The Lancet. 2006;367(9505):133-43.

68. Isanaka S, Aboud S, Mugusi F, Bosch RJ, Willett WC, Spiegelman D, et al. Iron status predicts treatment failure and mortality in tuberculosis patients: a prospective cohort study from Dar es Salaam, Tanzania. PLoS ONE. 2012;7(5):e37350.

69. Murray MJ, Murray AB, Murray MB, Murray CJ. The adverse effect of iron repletion on the course of certain infections. Br Med J. 1978;2:1113-5.

70. Gwamaka M, Kurtis JD, Sorensen BE, Holte S, Morrison R, Mutabingwa TK, et al. Iron deficiency protects against severe Plasmodium falciparum malaria and death in young children. Clin Infect Dis. 2012;54(8):1137-44.

71. Mounier-Jack S, Burchett HE, Griffiths UK, Konate M, Diarra KS. Meningococcal vaccine introduction in Mali through mass campaigns and its impact on the health system. Glob Health Sci Pract. 2014;2(1):117-29.

72. Tounkara B, Yalcouye I, Modjirom N. Programme Elargi de Vaccination: Evaluation de la campagne de vaccination contra la meningite avec le vaccin MenAfriVac, 2ème phase, dans les regions de Koulikoro, Segou et le District de Bamako au Mali in 2010. Geneva, Switzerland: Organisation Mondiale de la Santé, 2011.

73. Maslanka S, Gheesling L, Libutti D, Donaldson K, Harakeh H, Dykes J, et al. Standardization and a multilaboratory comparison of *Neisseria meningitidis* serogroup A and C serum bactericidal assays. Clin Diagn Lab Immunol. 1997;4(2):156-67.

74. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. Nutritional Methodology. 2004;134:3127-32.

75. Assessing the iron status of populations: including literature reviews: report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level. Geneva, Switzerland: World Health Organization/Centers for Disease Control, 2004 6-8 April 2004. Report No.

76. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Geneva, Switzerland: World Health Organization, 2011.

77. Perng W, Mora-Plazas M, Marin C, Villamor E. Iron status and linear growth: a prospective study in school-age children. Eur J Clin Nutr. 2013;67(6):646-51.

78. Bailey RL, West KP, Jr., Black RE. The epidemiology of global micronutrient deficiencies. Ann Nutr Metab. 2015;66 Suppl 2:22-33.

79. Bresnahan KA, Tanumihardjo SA. Undernutrition, the acute phase response to infection, and its effects on micronutrient status indicators. Adv Nutr. 2014;5(6):702-11.

80. Bhaskaram P, Arun Jyothi S, Visweswara Rao K, Narasinga Rao BS. Effects of subclinical vitamin A deficiency and administration of vitamin A as a single large dose on immune function in children. Nutr Res. 1989;9:1017-25.

81. Moore SE, Goldblatt D, Bates CJ, Prentice AM. Impact of nutritional status on antibody responses to different vaccines in undernourished Gambian children. Acta Paediatr. 2003;92:170-6.

82. Mullick S, Rusia U, Sikka M, Faridi MA. Impact of iron deficiency anaemia on T lymphocytes & their subsets in children. Indian J Med Res. 2006;124(6):647-54.

83. Oppenheimer SJ. Iron-deficiency anemia: reexamining the nature and magnitude of the public health problem. The Journal of Nutrition. 2001;131:616S-35S.

84. Hallquist N, McNeil L, Lockwood J, Sherman A. Maternal-iron-deficiency effects on peritoneal macrophage and peritoneal natural-killer-cell cytotoxicity in rat pups. Am J Clin Nutr. 1992;55:741-6.

85. Hershko C. Iron, infection and immune function. Proc Nutr Soc. 1993;52:165-74.

86. Nyakeriga AM, Troye-Blomberg M, Dorfman JR, Alexander ND, Ba¨ck R, Kortok M, et al. Iron deficiency and malaria among children living on the coast of Kenya. J Infect Dis. 2004;190:439-47.

87. Basta NE, Berthe A, Keita M, Onwuchekwa U, Tamboura B, Traore A, et al. Meningococcal carriage within households in the African meningitis belt: A longitudinal pilot study. J Infect. 2018;76(2):140-8.

88. MenAfriCar C. Meningococcal carriage in the African meningitis belt. Trop Med Int Health. 2013;18(8):968-78.

89. Ali O, Aseffa A, Omer AB, Lema T, Demissie TM, Tekletsion Y, et al. Household transmission of Neisseria meningitidis in the African meningitis belt: a longitudinal cohort study. The Lancet Global Health. 2016;4(12):e989-e95.

90. Cooper LV, Boukary RM, Aseffa A, Mihret W, Collard JM, Daugla D, et al. Investigation of correlates of protection against pharyngeal carriage of Neisseria meningitidis genogroups W and Y in the African meningitis belt. PLoS ONE. 2017;12(8):e0182575.

91. Diallo K, Trotter C, Timbine Y, Tamboura B, Sow SO, Issaka B, et al. Pharyngeal carriage of Neisseria species in the African meningitis belt. J Infect. 2016;72(6):667-77.

92. MacLennan J, Kafatos G, Neal K, Andrews N, Cameron JC, Roberts R, et al. Social behavior and meningococcal carriage in British teenagers. Emerg Infect Dis. 2006;12(6):950-7.

93. Vaughan AT, Gorringe A, Davenport V, Williams NA, Heyderman RS. Absence of mucosal immunity in the human upper respiratory tract to the commensal bacteria Neisseria lactamica but not pathogenic Neisseria

meningitidis during the peak age of nasopharyngeal carriage. J Immunol. 2009;182(4):2231-40.

94. Laver JR, Hughes SE, Read RC. Neisserial Molecular Adaptations to the Nasopharyngeal Niche. Adv Microb Physiol. 662015. p. 323-55.

95. Wessling-Resnick M. Iron homeostasis and the inflammatory response. Annu Rev Nutr. 2010;30:105-22.

96. Stevens GA, Bennett JE, Hennocq Q, Lu Y, De-Regil LM, Rogers L, et al. Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. The Lancet Global Health. 2015;3(9):e528-e36.

97. Sommer A. Preventing blindness and saving lives: the centenary of vitamin A. JAMA Ophthalmol. 2014;132(1):115-7.